

The effect of flax lignan complex supplementation and walking exercise on blood pressure of adults over 50 years old with prehypertension/stage I hypertension

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Abstract

This thesis evaluated supplementation of flax lignan complex (FLC) and walking exercise on blood pressure in older adults with prehypertension or stage I hypertension.

EXPERIMENT: This experiment assessed the effect of the flax lignan secoisolariciresinol diglucoside (SDG) in a FLC and walking exercise on blood pressure in older adults with prehypertension or stage I hypertension. Whey protein was used as supplementary placebo and stretching exercise was used as exercise placebo. **METHODS:** Twenty-five participants aged 50 years or older were recruited and randomized to receive either FLC or whey protein, concurrently with walking exercise or stretching exercise. Twenty-four-hour ambulatory blood pressure, body composition, and fasting serum blood glucose, triglycerides (TG), total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and c-reactive protein were measured before and after 8 weeks of interventions. **RESULTS:** Unexpectedly, there were significant time x supplement interactions for TG and HDL, as well as cholesterol:HDL ratio, in favour of whey protein. There were significant time x exercise interactions for nighttime diastolic blood pressure and nighttime mean arterial blood pressure in favour of stretching exercise. **CONCLUSION:** Eight weeks of SDG supplementation or walking exercise did not show any significant effect on blood pressure, serum glucose or serum lipid profile. Rather, whey protein and stretching exercise showed positive effects on cholesterol profile and nighttime ambulatory blood pressure, respectively.

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Also, I would like to express my appreciation to all participants who voluntarily committed their time to this project.

Dedication

I dedicate this thesis to my father, mother, brother, and my love, Seyoung Kim, who provided endless support, motivation and love.

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List of Abbreviations

ACE - angiotensin conversion enzyme

AMBP - Ambulatory blood pressure monitoring

AMPK - AMP-activated protein kinase

ALA - α -linolenic acid

aPWV - aortic pulse wave velocity

BP – blood pressure

cGMP - cyclic guanosine monophosphate

CHD - coronary heart disease

CL - chemiluminescence

CRP - C-reactive protein

CSEP - Canadian Society for Exercise Physiology

CVD – cardiovascular disease

DHA - docosahexaenoic acid

ED - enterodiol

EL – enterolactone

eNOS – endothelial nitric oxide synthase

EPA - eicosapentaenoic acid

FLC - flaxseed lignan complex

GLUT - glucose transporter protein

GTP - guanylate triphosphate

HDL - high-density lipoprotein

HR – heart rate

LDL - low-density lipoprotein

LPL - lipoprotein lipase

LV - left ventricular

MCP-1 - monocyte chemotactic protein-1

NO - nitric oxide

PMNL - polymorphonuclear leukocyte

PP - pulse pressure

RAAS - renin-angiotensin-aldosterone system

ROS - reactive oxygen species

SECO - secoisolariciresinol

SDG - secoisolariciresinol diglucoside

sICAM-1 - intercellular adhesion molecule-1

SOD - superoxide dismutase

TG - triglyceride

VCAM-1 - vascular cell adhesion molecule-1

WHO - World Health Organization

Chapter 1: Introduction and Literature review

1.1 Introduction

Hypertension has been studied very closely, being a major contributor to chronic diseases such as cardiovascular diseases, obesity, and diabetes. It is a top risk factor predicting cardiovascular diseases, and the prevalence of hypertension is expected to increase every year. The older adult population has a higher prevalence because of a sedentary lifestyle and the natural cardiovascular aging process. Despite many pharmacological treatments for hypertension, such as angiotensin conversion enzyme (ACE) inhibitor, calcium channel blocker, and diuretics, many prefer non-pharmacological approaches such as lifestyle modification. While low-fat, low-sodium diets with ingestion of enough fruit and vegetables have been suggested for dietary modification, supplementation of a bioactive product such as plant lignan has also been suggested as an alternative non-pharmacological approach for prevention or treatment of hypertension. Many research studies have proposed the possible health benefits from lignan supplementation; flaxseed has been highlighted as a ‘superfood’ because it is known as the richest source of plant lignan, or secoisolariciresinol diglucoside (SDG). At the same time, regular physical activity has been known for promoting cardiovascular health. Thirty or more minutes of moderate to vigorous physical activity for most days of the week is suggested as a sufficient volume for health benefits. Among many physical activities, walking is often suggested because of its simplicity and accessibility.

Through this thesis, I would like to explore the combined benefits of flax lignan and exercise for reducing blood pressure, especially in the older adult population with prehypertension or stage I hypertension.

The literature review introduces the prevalence and problem of hypertension, and possible non-pharmacological approaches (flax lignan supplementation and walking exercise)

and the mechanisms behind their potential benefits, as well as ambulatory blood pressure monitoring, which can provide more valuable information than conventional single time blood pressure measurement.

1.2 Hypertension

1.2.1 Prevalence of hypertension (Canada/worldwide) and impact on Society

Hypertension is one of the top risk factors predicting cardiovascular disease, which is a leading cause of death in many countries. Hypertension is also one of the main components of the metabolic syndrome. Metabolic syndrome is diagnosed when an individual has two or more conditions of either high plasma TG, high LDL, high blood pressure, low HDL, or high glucose level along with waist circumference larger than recommended (Genest et al., 2009). According to the World Health Organization (WHO), globally hypertension causes at least 45% of deaths from cardiovascular disease every year (WHO, 2013). In 2008, hypertension caused about 17% of total deaths in the world, and this is projected to increase to 24% by 2030 (WHO, 2013). The difference in prevalence of hypertension between countries may be due to factors such as geographic variation, genetic factors, or socioeconomic environment (Wolf-Maier et al., 2003). Often, lower income countries tend to have a higher prevalence of hypertension than high-income countries (Wolf-Maier et al. 2003, WHO, 2013), and blacks tend to have a higher percentage of hypertension compared to Caucasian (WHO, 2013). Canada and US have a lower prevalence of hypertension and have better controls for hypertension compared to other countries in the world (Wolf-Maier et al. 2003, Chow et al. 2013). Between 2012 and 2013, the prevalence of hypertension among Canadian adults was 22.6%, and 68.1% of them were controlling hypertension with medication (Padwal et al. 2016). One study projected that the prevalence of hypertension in Canada would go up to

29% by 2020 (Weaver et al. 2015). In Canada, healthcare spending for hypertension is about 10.2% of the total healthcare budget and is expected to rise even more. In 2010, the health care cost attributable to hypertension was estimated to be about 13.9 billion dollars and was expected to rise up to 20.5 billion dollars by 2020 (Weaver et al. 2015). In Alberta, the average health care cost for individuals with hypertension was more than 2 times higher than those who do not have hypertension in all age groups (Weaver et al. 2015). Therefore, detection and management of hypertension could contribute to less economic burden to individuals and society, as well as protection from other chronic diseases attributed to hypertension.

Hypertension is possibly the most important preventable factor of morbidity and mortality when managed properly. Hypertension is known as the top risk factor for chronic diseases such as ischemic stroke, type II diabetes, many cardiovascular diseases, and metabolic syndrome (Nerenberg et al., 2018). Hypertension prevalence is also increasing because of population growth, obesity, aging populations, and behavioral risk factors such as diet, alcohol, and lack of physical activity (Joffres et al. 2013; WHO. 2013). Also, these factors elicit additive effects on increasing cardiovascular risk. The prevalence of hypertension is strongly correlated with stroke and ischaemic heart disease mortality (Joffres et al. 2013). High blood pressure is a common sign of increased cardiovascular disease and associated with thickening of carotid intima and left ventricular wall, and diastolic dysfunction (Vasan et al. 2001). As blood pressure changes from optimal to high normal levels, the risk of cardiovascular disease and cumulative incidence increased in a stepwise manner (Vasan et al. 2001). A trend for sedentary lifestyle habits is causing people to be more susceptible to be hypertensive, and current food consumption patterns are tending to be westernized due to more access to animal and fat products and franchised food (Kearney

2010). A shift to high fat, sugar, and salt can cause an increase in health concerns such as overweight and diabetes (Kearney, 2010). Also, reduced physical activity increases the risk of metabolic syndrome, hypertension and cardiovascular diseases (Ford et al., 2005). According to the Canadian Society for Exercise Physiology, at least 150 minutes/week of moderate to vigorous exercise is recommended for adults (18 years – 64 years) and older adults (> 65 years) (CSEP, 2011). However, among all WHO countries, physical activity levels are decreasing, and this trend was more common in high-income countries (Hallal et al. 2012). Industrial and technology development in the modern era have caused an increase in sitting during work, as well as lower physically active level during regular daily life (e.g., driving, watching TV, playing computer, games, etc.). In 2012, only 31.1 % of adults were physically active worldwide (Hallal et al. 2012). Decreasing physical activity could be problematic because regular physical activity protects against many non-communicable diseases, which are often associated with hypertension. There is a dose-response relationship between physical activity and incidence of hypertension, and physical activities could reduce the risk of hypertension by 32% (Warbunton et al., 2010).

1.2.2 Classification of hypertension and its treatment

Hypertension can be divided into several stages depending on the systolic/diastolic blood pressure values. According to the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure, stage 1 is when an individual's systolic blood pressure is between 140-159 mmHg or diastolic blood pressure between 90-99 mmHg (Bakris et al., 2003). Often lifestyle modification is the most essential part of the treatments for individuals in this group (Nerenberg et al., 2018). Although under 140/90 is considered normal, 130-139/85-89 can be classified as high normal blood pressure or prehypertension. Treatment is not necessary at this point and arguably at stage I hypertension, and the

condition can be improved by changing lifestyle such as adding physical activity and/or modifying diet. However, in 2017, the American Heart Association raised the bar for the definition of stage I hypertension to 130-139/80-89, which made nearly half of the American adult population (46 %) become hypertensive (Whelton et al., 2017). For hypertension patients, physicians can provide one or more antihypertensive agents, such as thiazide diuretics, angiotensin-converting enzyme (ACE) inhibitors, Ca channel blockers, metformin, insulin, statins and aspirin, as well as some new interventions, such as renal denervation and carotid baroreceptor stimulation (WHO, 2013; Redon et al. 2016). Despite its wide usage, these pharmacological approaches can have side effects; therefore, after initial BP elevation, the American Heart Association recommends about 3-6 months of non-pharmacological therapy for prehypertensive or stage I hypertension adults, or about one month of combination of non-pharmacological and first line antihypertensive drugs for stage I hypertensive adults (Whelton et al., 2017).

Even though the risk of hypertension has been well-documented, and many different treatments are available, controlling hypertension is still a lifetime burden and not 100% controlled in some countries (Redon et al. 2016). The challenge to hypertension treatment can be inadequate primary prevention, faulty awareness of risk, lack of simplicity of the treatments, therapeutic inertia (the failure of health-care provider to initiate/intensify therapy), inadequate patient education, and an unsupportive healthcare system (Redon et al. 2016; Lebeau et al., 2014).

1.2.3 Risk of hypertension in older adults

The Canadian Hypertension Education Program sets the variety of recommendation from diagnosis to prevention/treatment. Although hypertension is generally defined as systolic blood pressure over 140 mmHg and/or diastolic blood pressure over 90 mmHg,

confirming hypertension need to be done in multiple steps by either using ambulatory blood pressure monitor or monitoring blood pressure with 2-4 additional visit to the clinic (Nerenberg et al., 2018). When ambulatory blood pressure was greater than 130/85 at the first and second visit, this can be defined as hypertension. Alternatively, when the office blood pressure measurement at the second or third visit was greater than 160/100 or 140/90, this can be defined as hypertension (Nerenberg et al., 2018). This multiple assessment is not only to make sure the hypertensive condition but also to eliminate the possible white coat hypertension, which does not require the pharmacological treatment. Further, regular physical activity, weight management, dietary habit changes (e.g. reducing consumption of alcohol, sodium, potassium, calcium, magnesium, and fat; increasing consumption of fruit and vegetables) are recommended to prevent and treat the hypertensive condition. (Nerenberg et al., 2018). From 2012 to 2013, hypertension was prevalent in 22% of Canadian adults between ages of 20 and 79, and its prevalence increased dramatically with age (Statistics Canada, 2014). Interestingly, hypertension prevalence is increased even in normal weight individuals after 40 years of age. About 17% of normal weight adults have hypertension, and this prevalence increases to 36% between ages of 60-79 years (Statistics Canada, 2014). Adults between ages of 40-59 years have 27% prevalence, and this goes up to 57% in between ages of 60-79 years (Statistics Canada, 2014).

Aging is associated with the following cardiovascular changes: cardiovascular structural remodeling, cardiovascular function, cardiovascular regulation, and physiological deconditioning (Lakatta, 2002). The cardiovascular system undergoes various changes at both the molecular and cellular level in the vascular bed and heart. Heart function is decreased by losing contractility and increased myocardial stiffness, while arterial vessels thicken, as a result of atherosclerosis, and stiffen from losing vascular tone regulation (Lakatta and Levy,

2003). Atherosclerosis itself also reduces blood vessel diameter, which causes increased peripheral resistance to blood flow. The development of lesions on the vessel wall leads to atherosclerosis, and this can continuously damage the vessel and grow the lesion to block the blood vessel (Davies, 2009). Older adults have increased arterial stiffness from hypertension, diabetes, atherosclerosis, and inflammation of blood vessels, especially when one has a sedentary lifestyle. Arterial stiffness is important in cardiovascular health because large elastic arteries buffer the hemodynamic impact from left ventricular (LV) stroke volume (Seals et al., 2009). Increasing vascular thickness, stiffness, left ventricular wall and left atrial size will raise blood pressure by increasing afterload of the heart, causing more demand for work to the heart (Lakatta, 2002). Possible mechanisms of vascular aging are by an increase in smooth muscle tone (Gao et al., 2014), endothelial dysfunction, increase in collagen and its cross-links, and fragmentation of elastin (Seals et al., 2009). Visceral fat is associated with higher aortic pulse wave velocity (aPWV), indicating higher aortic and vascular stiffness (Sutton-Tyrrell et al., 2001). It is important to note that even in healthy physically active men, arterial compliance decreases with aging. Aging-related vascular stiffness can be characterized by high systolic pressure and decreased diastolic pressure, widening the pulse pressure (PP), the difference between systolic and diastolic blood pressure (Steppan et al. 2011). However, the reduction in age-related arterial compliance can be delayed with exercise training (Tanaka et al., 2000).

1.2.4 Aging and development of hypertension

With aging, endothelium-derived nitric oxide (NO) function is also impaired. Nitric oxide plays a crucial role in controlling blood pressure by relaxing the smooth muscle of the blood vessel. NO is produced through a pathway involving NO synthase. This pathway could be activated by several molecules such as acetylcholine, serotonin, histamine, thrombin, ADP,

bradykinin, norepinephrine, calcium, estrogen, and shear stress from blood flow (Forte et al., 1997; Cignarella, 2001; Dimmeler, 1999; Tousoulis et al., 2012). Impaired NO function will affect total peripheral resistance, resulting in increased afterload on the heart, and this eventually increases blood pressure. Endothelial function is decreased with aging, and this phenomenon has sex differences; males tend to be more prone to rise in blood pressure in middle age, while premenopausal women are very protected from endothelial dysfunction due to high estrogen levels, which preserves NO bioavailability (Virdis and Taddei, 2012). Once females pass the menopausal period, the prevalence of endothelial dysfunction also increases significantly, and risk can even surpass that of males (Lima et al., 2012). Estrogen induces NO production via activation of endothelial nitric oxide synthase (eNOS) (Xia and Krukoff, 2004; Nevzati et al., 2015). NO production is reduced with aging, and oxidative stress is proposed as a main cause for this (Taddei et al., 2001). When the arteries of aged rats corresponding to 65 – 70-year-old humans were tested, decreased NO release from the endothelial cells was observed (Csiszar et al., 2002). Not only was NO production reduced but acetylcholine-induced vasodilation was reduced with age (Taddei et al., 2001).

Oxidative stress can produce reactive oxygen species (ROS), which contains an unpaired electron, causing cellular damage. Among many antioxidative enzymes, superoxide dismutase (SOD) plays a crucial role in regulating the oxidative stress from reactive oxidative species, such as superoxide (O_2^-). Superoxide dismutase reduces oxidative stress by reacting with non-paired electrons and converts reactive superoxide into oxygen or hydrogen peroxide. In the hypercholesterolemic state, endothelial function is seriously impaired because the activity of SOD is decreased, and nitric acid reacts with superoxide, which can degrade NO (Tousoulis et al., 2012; Cai and Harrison, 2000). ROS, such as superoxide, are produced by NAD(P)H oxidase, which could be stimulated by angiotensin II, thrombin,

platelet-derived growth factor, tumor growth factor A, or lactosylceramide, or mechanical stresses (Cai and Harrison, 2000). While oxidative stress increases, the anti-oxidant function of the endothelial cells is attenuated with aging, aggravating the vascular health of the aging individuals. Increased NADPH oxidase activity increases oxidative stress, and it is associated with risk of hypercholesterolemia, hypertension, and/or diabetes (Csiszar et al., 2002). Oxidative stress is a leading cause of impairment of the NO production pathway (Taddei et al., 2001). Reduction in NO bioavailability causes impaired NO-dependent vasodilation of blood vessels and eventually leads to hypertension.

Hypertension is caused by and/or further promotes inflammation in the endothelial cells, which leads to atherosclerosis. High-pressure blood flow can mechanically stimulate the endothelial cells, causing upregulation of gene expression of certain proteins such as intercellular adhesion molecule-1 (sICAM-1), and monocyte chemotactic protein-1 (MCP-1) (Chae et al., 2001). Both proteins play a role in increasing monocyte adhesion on the endothelial wall, followed by atherosclerosis development. There is a dose-dependent relationship between pulse pressure and sICAM, and increased blood pressure was highly associated with the level of IL-6, which plays a role in lesion development (Cae et al., 2001). Inflammation leads to activation of macrophages on vascular cells, damaging their endothelial function (Viridis et al., 2014). When the macrophage system was muted in an animal model, blood pressure elevation was better tolerated (Wenzel et al., 2011). The macrophage system, as well as T-cells and B-cells, could be increased not only by increased inflammation but the increased level of angiotensin II, which plays a critical role in increased blood pressure through the Renin-Angiotensin-aldosterone system (Harrison et al., 2012). Activation of the Macrophage system provokes further activation of NAD(P)H oxidase and cytokines, which generate ROS (Viridis et al., 2014). As mentioned earlier, increased ROS

cause production of adhesive protein and cytokines, reducing NO availability in endothelial cells; therefore, oxidative stress and inflammatory stress in vascular endothelial cells act in a cycle. In human cross-sectional studies, sICAM-1, IL-6, CRP, TNF- α were highly associated with elevated blood pressure (Virdis et al., 2014). In the hypertensive condition, elevated C-reactive protein (CRP), a strong predictor of cardiovascular disease, is observed, but associations with hypertension could be different between ethnic groups and even between sexes (Ghanem and Movahed, 2006). Although inflammatory markers and hypertension are highly associated with each other, pharmacologically targeting the inflammatory markers does not necessarily reduce blood pressure; therefore, targeting inflammatory markers with pharmacological treatment remains questionable (Ghanem and Movahed, 2006). CRP reduction can be achieved by lifestyle modification, such as increased physical activity, quitting smoking, and changing dietary habits (Esposito et al., 2003).

1.3 Physical activity

1.3.1 Recommended physical activity (for older adults)

Physical activity is important for preventing cardiovascular disease. Fitness level is inversely associated with blood pressure (Kokkinos, 2009). Not only does physical activity slow down the aging of the cardiovascular system, but it also improves impaired cardiovascular health. When an exercise intervention was introduced to hypertensive individuals, blood pressure was significantly reduced (Kokkinos, 2009). The Canadian Society for Exercise Physiology suggests adults 18 years and older accumulate 150 minutes of moderate to vigorous intensity aerobic exercise per week, in bouts of 10 minutes or more, to reduce the risk of premature death, coronary heart disease, stroke, hypertension.. (Canadian Society of Exercise Physiology, 2011). It is recommended that Stage 1

hypertension patients achieve moderate levels of physical activity, such as 30-40 minutes walking, most days of the week (Kokkinos et al. 2009). Similar recommendation is given by systematic review from Warburton et al. that 30 minutes or more of moderate to vigorous exercise on most days of the week is recommended to reduce the risk of hypertension (Warburton et al., 2010). However, some studies showed blood pressure drops even with exercise done at a lower intensity (Kokkinos et al., 2009). Sedentary middle-aged and old men had significantly less arterial compliance than those who had a history of endurance training, but with a 3-month exercise intervention, arterial compliance of untrained males significantly increased (Seals et al. 2009). Sedentary lifestyle leads to high triglyceride (TG), low-density lipoprotein (LDL) level, and low high-density lipoprotein (HDL), which are all associated with risk of cardiovascular disease. While sedentary lifestyle can lead to decreases in lipoprotein lipase activity, causing increased plasma lipid level and reduced HDL level, and decrease in glucose transporter protein (GLUT), physical activity can restore the activity of these two critical proteins involved in fat and glucose metabolism (Tremblay et al., 2010).

1.3.2 Physiological changes from physical activity

An active lifestyle helps to retain cardiovascular function with aging. During “healthy aging”, a sedentary lifestyle causes decreased left ventricular compliance, but with regular endurance training, the ventricular compliance could be preserved, leading to less chance of heart failure in older adults (Arbab-Zadeh et al. 2004). When older adults (~69 years old) were divided into 4 groups (i.e. sedentary (< once/week), casual (2-3 sessions/week), committed (4-5 sessions/week), masters athletes 6-7 sessions/week)) depending on exercise dose during their lifetime, those who were in the committed group or masters athletes group for at least 25 years showed enhanced O₂ transport and utilization during exercise (Arbab-

Zadeh et al., 2004). A high frequency of lifelong exercise was effective to limit the reduction in cardiovascular function with aging. Also, frequent lifelong exercise was associated with larger total blood volume, as well as larger left ventricular mass and could prevent decreased LV compliance and distensibility (Carrick-Ransom et al., 2014; Bhella et al., 2014). Further, trained older adults had improved systolic function with reduction in aortic stiffness and ventricular resistance, improved ventricular-arterial coupling, and enhanced β -adrenergic sensitivity (Carrick-Ransom et al., 2014). Low dose of casual exercise (2-3 sessions per week) did not prevent the decrease in left ventricular compliance and distensibility (Bhella et al., 2014). The adaptation from life-long exercise could be due to increased mitochondria and oxidative enzyme activity, vasodilator response, and enhanced blood distribution to working muscle (Carrick-Ransom et al., 2014). Regular aerobic training induces physiological adaptation of muscle cells. To meet the demand from exercise, cardiomyocytes grow longitudinally, which is often referred to as “eccentric hypertrophy” (Wisloff et al. 2009). Older athletes who participated in endurance racing for 10 years showed lower HR, lower resting blood pressure, and greater aerobic capacity (VO_{2max}) than sedentary older adults (Albab-Zadeh et al. 2004). However, even sedentary older adults could significantly improve their HR, and blood pressure after an exercise intervention (Molmen et al., 2012). Although older adults have smaller LV volume than young people due to atrophy, exercise interventions can improve their LV volume and end diastolic volume by eccentric hypertrophy (Molmen et al., 2012). This indicates that the main cause of cardiac atrophy is inactivity or age-derived atrophy, and this could be minimized by exercise training.

1.3.3 Benefit from physical activity

With aging, endothelium-derived nitric oxide function is also impaired. Knowing that NO is an important vasodilator at the microvascular level, impaired NO function will affect

total peripheral resistance, resulting in increased afterload on an individual's heart. The sedentary older adult population has less NO response to both heat and ACh mediated vasodilation. However, with 24-weeks of exercise training, unfit older adults normalized NO mediated microvascular function (Black et al., 2008). With exercise, eNOS gene and protein expression was increased in old rat arteries and mammalian arteries (Seals et al., 2009). Also, oxidative stress with aging is reduced with habitual aerobic exercise (Seals et al., 2009). Another important mechanism of enhancing endothelial function is the shear stress from exercise (Di Francescomarino et al., 2009). During exercise, blood flow exerts force on the arterial wall, which stimulates endothelium, resulting in upregulation of endothelial nitric oxide synthase, as well as superoxide dismutase and glutathione peroxidase, which reduces oxidative stress from reactive oxygen species (Di Francescomarino et al., 2009). When old sedentary and exercising animals and humans were compared, sedentary groups had greater oxidative stress and inflammation, reduction in NO production and increase in structural remodeling, such as increased collagen and decrease in elastin (Santos-Parker et al., 2014). Some of these effects, such as oxidative stress, inflammation, NO production and collagen formation were reversed after an exercise intervention (Santos-Parker et al., 2014).

Exercise training alters the ability of blood vessels to vasodilate by altering blood vessel structure. Arteries adapt to exercise by functional vascular adaptation (activation of eNOS, increased NO), followed by vascular remodeling (Tinken et al., 2008). In this study, lower limb strength training initially increased the endothelial function of blood vessel by providing repetitive shear stress on blood vessels, enhancing NO production, then exercise increased blood flow and hemodynamics, which enhances the structural change of blood vessels (Maiorana et al., 2003). While several weeks of 'short-term' exercise training enhances NO production for improved vasodilation, long-term exercise alters the vascular

tone via remodeling, for which NO plays a critical role. In another study using a mice model, eNOS function was knocked out to eliminate NO induced vasodilation. These mice showed thickening of the blood vessel wall and luminal remodeling was impaired (Rudic et al., 1998). NO stimulates vessel remodeling by inhibiting smooth muscle proliferation or smooth muscle mitogen, which reduces the diameter of blood vessels (Rudic et al., 1998). The importance of NO in blood vessel adaptation clearly links to the pre-mentioned lower NO bio-availability in hypertension, and atherosclerotic conditions.

Habitual exercise can protect against the negative changes on the vessels caused by CVD risk factors. In middle-aged adults, there is a significant association between an increase in the baseline level of physical activity and elevation of HDL, reduction in the TG for both sexes, in addition to reduction in LDL in females (Monda et al., 2009). Exercise can reduce the age-associated vascular stiffness by increasing NO production and reducing oxidative stress (Seals et al., 2009). When sedentary adults underwent a moderate intensity exercise intervention for 6 months, their fat clearance, lipoprotein lipase (LPL), and insulin sensitivity were improved (Duncan et al., 2003). When diabetic male participants were subjected to 12-16 weeks of moderate intensity (50-60% $\text{VO}_{2\text{peak}}$) endurance training, there was an increased HDL/LDL ratio and apolipoprotein A-I (A-I)/apolipoprotein B (apo B) ratio, with decrease in LDL and apo B level (Laaksonen et al., 2000). Improved lipid profile from exercise can be explained by increase in fat oxidation via upregulating oxidative enzymes, which improves cholesterol profile (e.g. increase in HDL and reduction in LDL) (Goodpaster et al., 2003; Mann et al., 2014). Both HDL and LDL are critical in controlling the circulating lipid level; LDL transports lipid into the blood circulation, while HDL delivers lipids back to the liver. Low HDL level is one of the significant risk factors for cardiovascular diseases especially in older adults (Curb et al., 2004), and is considered as one component of

lipid profile that improves due to the physical activity (Mann et al., 2014).

Walking exercise has been clearly shown to have positive effects on blood pressure in a wide range of the population, in both hypertensive and normotensive individuals (Lee et al., 2010). Therefore, walking exercise has been suggested as one of the first exercise options to start, because of its accessibility and convenience for performance.

1.4 Flaxseed

1.4.1 Flaxseed production in Canada/worldwide

Canada produces about 40 % of flaxseed worldwide, and other countries such as China, US, and India account for another 40% of global flaxseed production (Agricultural marketing resource center, 2013). In 2016, 579 thousand tons of flaxseed was produced from Canada, and Saskatchewan produced about 82% of total Canadian flaxseed (Canadian grain commission, 2016, <https://www.grainscanada.gc.ca/flax-lin/harvest-recolte/2016/hqfl6-qrl16-2-en.htm?wbdisable=true>).

Flaxseed has been highlighted recently as a great source of bioactive components, such as α -linolenic acid, lignans, and dietary fiber (Kajla et al., 2015). Flaxseed is composed of protein, fat, total dietary fibre, carbohydrates, folic acid, and other mineral and micronutrients such as vitamin A, and E (Kajla et al., 2015). Due to benefits from these nutritional components, interest in flaxseed supplementation has been increasing.

1.4.2 Omega-3 fatty acid component of flaxseed

Flaxseed is known as the best non-fish omega 3 fatty acid source (Kajla et al., 2015). The omega 3 fatty acid, alpha-linolenic acid, from flaxseed has anti-inflammatory, anti-

thrombotic and anti-arrhythmic effects (Kajla et al., 2015). These fatty acids of the flaxseed have been studied closely for how they provide health benefits when ingested.

Omega 3 fatty acids are inversely associated with cardiovascular disease; there is lower CVD incident rate in those consuming more fish than those who do not (Ruxton et al. 2004). Omega 3 fatty acid components in flaxseed exert beneficial effects in many different ways. Omega 3 fatty acids activate PPAR α , a transcription factor for proteins playing a crucial role in β -oxidation and lipoprotein metabolism and PPAR γ , responsible for adipocyte differentiation and production of inflammation mediators, such as TNF α and IL-6 (Calder, 2012). PPAR γ also can improve insulin sensitivity, therefore providing an improvement in glucose profile (Calder, 2012). Through these mechanisms, omega 3 fatty acids can improve lipid profiles and protect against inflammation of blood vessels. Plant omega 3 fatty acids, such as α -linolenic acid and stearidonic acid, are precursors of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which directly act on PPAR α and PPAR γ for beneficial effects (Calder, 2012; Surette, 2008). When omega 3 fatty acids (EPA/DHA) were given to hyperglycemic patients, these fatty acids decreased serum TG level by 20-50% (Bays et al. 2008). Healthy Japanese men administrated flaxseed oil showed lower total cholesterol and LDL level than a control group after a 12-week intervention, but no change in triglyceride level (Kawakami et al. 2015). Flaxseed oil supplementation showed larger increases in serum omega 3 fatty acids than olive oil supplementation, indicating that flaxseed oil can be a good source of omega 3 fatty acids (Grindel et al. 2013). When healthy adults ingested omega 3 fatty acid through flaxseed oil, fish oil, or hempseed, there was no significant change in lipid parameters, such as total cholesterol, HDL, and LDL level (Nalini et al. 2008).

1.4.3 Dietary fibre component of flaxseed

Dietary fiber of the flaxseed is also a well-known component protecting against

cardiovascular diseases. When adult women between 37–64 years old were epidemiologically analyzed, dietary fiber intake reduced the risk of coronary heart disease (CHD) (Wolk et al. 1999). In Japanese women aged between 45 and 65 years, total fiber intake was inversely associated with cardiovascular diseases (Kokubo et al. 2011). Dietary fiber also can bring down blood pressure, total cholesterol and LDL levels (Rees et al. 2013; Hartley et al. 2016). Some mechanisms for how dietary fiber can bring down cholesterol and blood pressure have been suggested. Soluble fiber can inhibit fatty acid formation in the liver, alters the intestinal mobility, slows down micronutrition absorption, improves insulin sensitivity and increase satiety, leading to lower food ingestion (Brown et al. 1999; Hodgson et al. 2010).

Intake of flaxseed improves blood pressure, lipid profile, and other conditions related to metabolic syndrome (Rodriguez-Leyva et al., 2011; Cornish et al., 2009; Park et al., 2012). In the FLEX-PAD study by Rodriguez-Leyva et al. (2013), 110 hypertensive patients took 30 g of milled flaxseed for 6 months, and the mean systolic and diastolic blood pressure (\pm SD) of the flaxseed group was significantly reduced from $143\pm22/77\pm9$ mmHg to $136\pm22/72\pm11$ mmHg, while the placebo group had no changes in blood pressure. At the same time, beneficial omega-3 lipids, such as serum α -linolenic acid (ALA), eicosapentaenoic acid (EPA), and lignan components, such as enterolactone and enterodiol, were significantly increased after the supplementation. A small dose of flaxseed was sufficient to improve serum α -linolenic acid, enterodiol (ED) and enterolactone (EL) levels. Omega-3 fatty acids, especially ALA, EPA, and DHA, are well known for providing cardiovascular protection, through lowering blood pressure and resting heart rate (Mozaffarian and Wu. 2012). As low as 10g/day of flaxseed intake resulted in significantly increased circulating ALA, while at least 30g/day of flaxseed intake was needed to see the conversion of ALA to EPA (Edel et al. 2015). ALA is inversely associated with cardiovascular disease events, such as coronary heart

disease and congestive heart failure, in older adults (Pan et al. 2012; Fretts et al., 2014). ALA provides cardiovascular protection through different mechanisms; lowering LDL and blood pressure, increasing arterial compliance, reducing platelet aggregation, as well as reducing inflammation (Baker et al. 2016). Flaxseed supplementation successfully improved plasma cholesterol level, glucose concentration, and blood pressure (Edel et al., 2015).

Many research studies have proposed that flaxseed supplementation has health benefits such as protection against diabetes, metabolic syndrome, cardiovascular diseases, oxidative stress, inflammation, and even some types of cancers (Adolphe et al. 2010). Although there are many clinical studies using flaxseed supplementation, flaxseed is often treated differently; e.g., whole flaxseed, ground flaxseed, flaxseed hull, flaxseed oil, or flaxseed lignan. Therefore, the component or combination of components of flaxseed which exerts beneficial effects is not clear.

1.5 Secoisolariciresinol diglucoside (SDG)

1.5.1 Benefit from SDG

Phytoestrogens, such as lignans and isoflavones, have been highlighted as beneficial compounds from plants. These naturally occurring compounds have structural similarity with estrogen, and many studies have investigated how phytestrogens elicit beneficial effects on the human body, especially in pathological conditions. Typical lignans found in foods are matairesinol, pinoresinol, medioresinol, lariciresinol, sesamin, syringaresinol, secoisolariciresinol, secoisolariciresinol diglucoside, which is glycosylated secoisolariciresinol (Yoder et al. 2015). While flaxseed is the richest known source of lignans, lignans are also found in seeds such as sesame seeds, cloudberry seeds, hemp seeds, cereal

grains such as rye, wheat, barley, oats, and other vegetables, as well as tea, milk, and juice (Yoder et al. 2015). Among the beneficial nutrients in flaxseed, the plant lignan secoisolariciresinol diglucoside (SDG) is thought to exert the most beneficial effects on the human and animal body systems (Adolphe et al., 2010). Lignan is a bioactive compound with many beneficial effects such as antioxidant, protection against cancer, estrogenic, anti-estrogenic effects.. Food sources with the most concentrated lignans are flaxseed and sesame seed (Lampe et al. 2006). There is a possible dose-response relationship with SDG administration. The typical daily dose of SDG in many studies is 600 mg. When administering different SDG doses (300 mg vs. 600 mg per day), 600 mg showed significant reduction in total blood cholesterol and LDL cholesterol level when compared with placebo, while 300 mg only showed a difference when compared with the baseline (Adolphe et al., 2010). Also, when comparing between purified SDG and flax lignan complex, both supplements showed significant beneficial effects. When rabbits were fed one of the flaxseeds with low α -linolenic acid, flaxseed oil, flaxseed lignan complex, or SDG, flaxseed lignan complex and SDG reduced the development of hypercholesterolemic atherosclerosis by 73 % and 34 %, indicating that the lignan content exerts beneficial effects in cholesterol reduction, rather than α -linolenic acid contents (Prasad, 2009). High blood cholesterol promotes atherosclerosis and causes narrowing of arteries, which increases total peripheral resistance. It increases afterload of the heart leading to increased blood pressure. Thus, blood pressure lowering effects can be anticipated from a reduction in cholesterol and LDL. The effect of high cholesterol and high blood pressure synergistically interact to increase the risk of CHD (Satoh et al., 2015). Previous studies support that SDG at a dose of 600 mg/day can improve hyperglycemia, dyslipidemia, blood pressure, central obesity, prothrombotic state, inflammation, and low-density lipoprotein oxidation (Adolphe et al., 2010; Satoh et al., 2015).

1.5.2 Metabolism of SDG and effects of its metabolites

The biological mechanism of how SDG exerts its beneficial effect is not fully understood, but it is thought to improve or protect against cardiovascular disease, diabetes, the metabolic syndrome, cancer, oxidative stress and inflammation (Adolphe et al., 2010). Once ingested, SDG is converted to secoisolariciresinol (SECO) by bacteria in the human colon to be absorbed into the circulatory system. SECO is then converted to enterodiol (ED) by dihydroxylation and demethylation. Enterodiol is oxidized to become enterolactone (EL) (Kajla et al., 2015). People with higher serum EL concentration had 58.8 % lower risk of acute coronary events compared with those with lower concentration, and this led to the EL level being used as biomarker to assess the risk of CVD (Vanharanta and Voutilainen, 1999). Enterolactone may protect against CVD through oxidative stress reduction and estrogenic effects; however, further studies are required because the effect from ingested fibre cannot be ruled out (Vanharanta and Voutilainen, 1999). ED and EL are structurally similar to oestrodinol and can bind to estrogen receptors to exert their effect (Adolphe et al., 2010). Thanks to its structural similarity to endogenous estrogen, SDG may exert either estrogenic or anti-estrogenic effect by binding to estrogen receptors. Whether SDG acts as an estrogen agonist or a competitor of estrogen depends on the systemic concentration of estrogen (Moree et al., 2011). Studies suggest that change in estrogen level before and after the menopause and the risk of cardiovascular disease are closely related. Premenopausal females get benefits from a high level of estrogen, and this effect is diminished once females experience menopause, which drops the estrogen level and causes an increased risk of CVD. An earlier observational study suggested that phytoestrogens, such as isoflavone and plant lignan, can act on the estrogen receptor to provide the beneficial effect in postmenopausal participants (Van der Schouw et al. 2002). Phytoestrogen may exert its beneficial effect through reducing response

to vasoconstriction, protecting endothelial cells against oxidative stress, as well as suppressing vascular cell adhesion molecule-1 (VCAM-1), which is involved in atherosclerosis development (Chin et al., 2001; Koh et al., 1999). However, another study suggested the plant lignan from flaxseed did not improve any endothelial function of postmenopausal women after 6 weeks of intervention (Hallund et al. 2006). In another study targeting both male and female adults, only males showed an improvement in diastolic blood pressure after 6 months of flaxseed lignan complex (FLC) supplementation (Cornish et al. 2009). From the rat model, researchers determined that SDG can exert cardiovascular protection by acting as an anti-ACE inhibitor or/and an agent that activates guanylyl cyclase, in a mechanism that dilates the blood vessels to reduce peripheral resistance and systemic blood pressure (Prasad, 2013; Prasad, 2005). Oxidative stress is also associated with increase in blood pressure, and as an individual becomes older, one's anti-oxidant capacity is reduced. SDG is a highly potent anti-oxidative agent, and this is another way it may provide beneficial effect (Silva et al., 2013).

1.5.3 Mechanisms of action

SDG reduced blood pressure in a dose-dependent manner in a rat model (Prasad, 2009). Systolic, diastolic, and mean arterial pressure decreased with increasing dose up to 10 mg/kg and reached a plateau after that point. SDG works as a long acting hypotensive agent by increasing guanylate cyclase enzyme activity (Prasad, 2009). Although the exact mechanism is not fully understood, SDG seems to reduce blood pressure in a similar manner to nitrovasodilators (Prasad, 2004). SDG exerts its BP lowering effect on the L-arginine-nitric oxide pathway. Vascular endothelial cells release nitric oxide, synthesized from L-arginine. Nitric oxide, then activates guanylate cyclase to convert guanylate triphosphate (GTP) to

cyclic guanosine monophosphate (cGMP). Vascular smooth muscle relaxes by action of cGMP. When rats were pre-treated with guanylate cyclase inhibitor, SDG-induced blood pressure reduction was attenuated, indicating that the SDG may directly act on guanylate cyclase activity (Prasad, 2004). An alternative mechanism for the hypotensive effect of SDG is an inhibition of angiotensin-conversion enzyme (ACE) (Prasad, 2013). This hypothesis stems from the idea that other plant phytestrogens, such as isoflavone, are inhibitors of ACE (Rupasinghe and Balasuriya, 2011). The renin-angiotensin-aldosterone system (RAAS) is one of the regulatory pathways to control blood pressure by liver and kidney. Angiotensin II is a peptide that plays an important role in increasing blood pressure, and ACE plays a critical role in converting angiotensin I to angiotensin II, which directly acts as a vasoconstrictor. Phytoestrogens, including SDG, inhibit the action of ACE. In a rat model, when angiotensin I was injected with or without SDG, rats treated with SDG showed long acting hypotensive effects for both systolic and diastolic blood pressure (Prasad 2013). These mechanisms were observed in an animal model, and it is not yet clear how lignan works in the human body. However, most studies propose a positive effect on human health.

SDG-fed mice had a reduction in fat accumulation in hepatocytes and reduced fasting glucose and insulin level after 16 weeks of SDG feeding (Sun et al., 2016). The reduction in fat storage in the liver by SDG is thought to occur through activation of AMP-activated protein kinase (AMPK) (Sun et al., 2016). Activation of hepatic AMPK will increase the β -oxidation of fatty acids and inhibit lipogenesis (Browning and Horton, 2004). In another study, lignan extracted from *Schisandra chinensis* fruit was fed along with a high fat diet to hamsters, and a significant anti-hyperlipidaemic effect was demonstrated through increased energy metabolism, resulting in reduced serum cholesterol, triglyceride, LDL, and inflammation (Liu et al., 2015).

Increased oxidative stress is another important cause of hypertension. Since anti-oxidative function becomes poorer with aging, older adults can benefit from taking SDG supplementation regularly because SDG has anti-oxidant properties. Metabolites of SDG, such as SECO, ED, and EL exert antioxidant activities more than SDG itself by reducing lipid peroxidation, deoxyribose oxidation and DNA strand breakage (Adophe et al., 2010). SDG and its metabolites also provide antioxidant activity by reducing the formation of polymorphonuclear leukocyte (PMNL) and chemiluminescence (CL). PMNL and CL increase oxidative stress of the vascular system by generating reactive oxygen species (ROS), such as O_2^- , H_2O_2 , hydroxyl radical ($\bullet OH$) and single oxygen (1O_2). (Prasad, 2009). The anti-oxidant properties of SDG may need to be investigated in greater depth because as individuals age, antioxidant capacity is diminished.

Although many studies support the idea that flaxseed lignan helps to reduce cardiovascular risk factors, a study by Hallund et al. (2006) did not find any significant difference in lipid profile between a placebo group and a lignan supplement group. However, considering this study targeted healthy subjects, SDG might show its risk factor lowering effect better if the subjects had had higher baseline level of risk factors than Hallund's study (Barre et al., 2012).

1.6 Ambulatory blood pressure monitoring

1.6.1 What is ambulatory blood pressure monitoring?

In most cases, a simple sphygmomanometer is used to measure blood pressure; however, this technique has limitations because it is only used to measure single time point blood pressure. Ambulatory blood pressure monitoring (ABMP) is an alternative blood

pressure measurement, using a blood pressure cuff (wired or wireless) linked with an automated device that periodically inflates the BP cuff for measurement. Ambulatory blood pressure monitoring allows the researcher and clinician to observe blood pressure over a continuous time course, usually for 24 hours.

1.6.2 When can AMBP be used?

There are three possible incorrect diagnoses from conventional single time blood pressure measurements. These errors occur when in-office and out-the-office blood pressure are not matched: White coat hypertension (patients with hypertension in the office environment but normotensive out-of-office), masked hypertension (normotensive at office but hypertensive outside the office environment), and sustained hypertension (hypertension in both environments) (Zhang et al., 2015). Masked hypertension carries a risk equivalent to sustained hypertension, and white coat hypertension carries a risk equivalent to normotension (Boggia et al., 2014). White coat hypertension is defined as blood pressure over 140/90 mmHg when measured in the clinic, but below 135/85 mmHg when measured in non-clinical settings (O'Brien et al., 2000). This phenomenon could be present in as high as 15-30% of people with high blood pressure, can cause socioeconomical disadvantage (e.g. monetary cost, insurance disadvantage), and unnecessary lifelong drug prescription (Franklin et al., 2013). This often happens in older adults or pregnant women. A simple transient adrenergic response due to the stress in a clinical situation could create the blood pressure difference (Shahbazian et al., 2013). Those who showed white coat hypertension had significantly higher sympathetic hyperactivity than normotensive people, causing blood pressure rise in the clinical setting (Smith et al., 2002). Since blood pressure in the office environment appears to be higher than actual pressure, white coat hypertension can overestimate the individual's

blood pressure, and possibly leads to excessive treatment (O'Brien et al., 2000). In a study targeting resistant hypertensive patients (remaining at $\geq 140/90$ even with anti-hypertensive agent), there was a large gap between office blood pressure and ambulatory blood pressure (Banegas et al. 2009). In this study, office systolic blood pressure was 28.4 mmHg higher than daytime ambulatory systolic blood pressure, and office diastolic blood pressure was 11.2 mmHg higher than daytime ambulatory diastolic blood pressure. Because such differences between two types of measurement can create critical misdiagnosis of patients, ambulatory blood pressure monitoring may insure whether a patient's blood pressure reading is accurate.

1.6.3 Advantage of ambulatory blood pressure monitoring

To overcome the disadvantages of conventional single time point blood pressure measurement, ambulatory blood pressure monitoring is often adopted. Ambulatory blood pressure monitors provide continuous blood pressure data during a given period. In addition, the data set can be broken down into certain time periods, for example, daytime and night time. Looking at night time blood pressure during sleeping could be valuable because it can determine whether the individual is a "dipper" (BP falls during the night) or a "non-dipper" (BP does not fall during the night). There is a strong association between non-dipper night time blood pressure with cardiovascular morbidity and mortality, and non-dipper blood pressure is a risk factor for target organ damage (Mahabala et al., 2013; Turner et al., 2015). Diabetic mellitus patients often have an altered circadian blood pressure profile, so blood pressure measurement at multiple points is important (Draman et al., 2015). Ambulatory blood pressure data provides valuable information for patients with obstructive sleep apnea. The severity of the sleep disorder is linearly associated with blood pressure, and the patient's blood pressure often rises during sleep time (Delsart et al., 2015). Higher nighttime

ambulatory blood pressure has a higher relative risk of cardiovascular death of 1.30, compared to lower night time ambulatory blood pressure whose relative risk remained below 1.00 (Dolan et al. 2005). Further, for patients subjected to hypertensive agent administration, ambulatory blood pressure data may provide valuable data such as acute change in blood pressure pattern after the drug ingestion. Although there are limitations to ambulatory blood pressure monitoring, such as cost and discomfort, hypertension guidelines from many countries recommend the use of an ambulatory monitor (Turner et al., 2015). Ambulatory blood pressure monitoring can not only eliminate the possible wrong diagnosis from white coat hypertension or masked hypertension, its data itself has a high association with the risk for cardiovascular disease (Verdecchia et al. 1998). Widening ambulatory pulse pressure, the difference between ambulatory systolic blood pressure and diastolic pressure, has been proposed as one of the risk factors for cardiovascular disease (Verdecchia et al. 1998). Total and fatal cardiovascular events were increased as ambulatory pulse pressure increased. Even after controlling for age, gender, LV hypertrophy, cholesterol, diabetes, smoking, white coat hypertension and non-dipper status, ambulatory PP remained significantly prognostic (Verdecchia et al. 1998). Ambulatory blood pressure showed steeper relationship with cardiovascular morbidity and mortality than office blood pressure, indicating that it may act as a better predictor for CDV risk (Mancia and Verdecchia, 2015).

Although studies propose an advantage of ambulatory blood pressure measurement over conventional blood pressure measurement, Hypertension Canada still categorizes it as Grade C, which indicates a lower level of validity and precision (Nerenberg et al., 2018).

1.7 Proposed problem

Although many studies support a beneficial effect of flaxseed supplementation and

regular walking exercise, the results are often mixed, and this is because of the inconsistency of flaxseed supplement type and targeting of different population groups. Therefore, in this study, I attempted to focus on the effect from the lignan component of flaxseed, with regular walking exercise, targeting only older adults with prehypertension or stage I hypertension but otherwise healthy. Also, knowing the possible limitation of conventional single time blood pressure measurement using a sphygmomanometer, ambulatory blood pressure monitoring was used to investigate the possible blood pressure change across different times of the day.

1.8 Hypothesis

Flax lignan complex (FLC) supplementation to prehypertensive/stage-1 hypertensive people will reduce ambulatory blood pressure, blood glucose and lipid profile after 8 weeks, compared to a placebo group.

Walking exercise for prehypertensive/stage-1 hypertensive people will reduce ambulatory blood pressure and improve blood glucose and lipid profile after 8 weeks, compared to a placebo exercise group (i.e. stretching).

FLC supplementation and walking exercise will show additive effects on ambulatory blood pressure, blood glucose and lipid profile.

Chapter 2: Methods and Results

2.1 Subjects and Methods

The protocol was registered in clinicaltrials.gov as NCT02391779. The study was approved by the ethics board of the University of Saskatchewan (BIO 194-14) and all participants signed a consent form prior to participation.

2.2 Participants

Twenty-five men and women over 50 years old were recruited from the general population by newspaper advertisement and posters on community boards. All except one were residents of Saskatoon, Saskatchewan, Canada, and one was from Prince Albert, Saskatchewan, Canada. For study inclusion, participants were required to be 50 years of age and over, having high normal blood pressure (130/85 – 139/89) or stage I hypertension (140/90 – 159/99) with the capability of walking unaided up to 30 minutes. Subjects were excluded from the study if they 1) were currently residing in a long-term care home; 2) had unstable diabetes or diabetic condition taking insulin; 3) had or had had cancer in the past 2 years; 4) had significant liver or other gastrointestinal disorders or inflammatory bowel disease; 5) had significant kidney disorder; 6) had taken oral antibiotics in the past three months; 7) had history of unstable or severe cardiac disease; 8) had heart attack or stroke in the past 2 years that severely affected physical mobility; 9) had other unstable medical diseases or conditions, such as pulmonary disorder, epilepsy, genitourinary disorder; 10) had migraine with aura within last one year; 11) were diagnosed with a bleeding condition or at risk for bleeding; 12) had significant immune compromise; 13) were using hormone replacement therapy, except thyroid medication, estrogen tablet/creams; 14) were using blood pressure medication and/or diuretics; 15) were using flax seed supplement; or 16) were participating in any other clinical trial within one month prior to randomization. A sample

size of $n = 17$ per group for flax seed supplementation and $n = 42$ per group for exercise training were calculated for adequate power by using previous research (Cornish et al. 2009; Fagard and Cornelissen, 2007) with an assumption that there would be an additive effect of flax lignan supplementation and exercise training. Compared to the control group, flax lignan supplementation and exercise training were expected to reduce diastolic blood pressure by 8 mmHg and 5 mmHg respectively with a standard deviation of 8 mmHg, alpha of 0.05, and power of 0.80. For 2 x 2 factorial ANOVA study design, a total of 100 participants was needed to be recruited and 25 participants would be assigned per group (i.e. for an exercise main effect and to account for participants who were expected to be lost to follow-up).

2.3 Methods

This clinical trial was registered (NCT02391779) at ClinicalTrials.gov. During the first visit, participants went through baseline measurements. Baseline measurements included height, weight, waist circumference, 24 h ambulatory blood pressure, Dual energy x-ray absorptiometry (DEXA, Hologic® Discovery QDR series) whole body scanning for body composition, food frequency questionnaires, and Physical Activity Score for the Elderly (PASE) questionnaires. Blood pressure was measured using a sphygmomanometer at standing and laying positions. Waist circumference was measured at the level right above the iliac crest. DEXA scanning measured body composition and bone mineral density, performed by certified technicians. During a 6-minute walking test, participants were asked to walk back and forth as fast as they could on a 30 m long track for 6 minutes. Before and after the walking, participants were asked about their fatigue level and shortness of breathing using the BORG scale. The total distance each participant walked was recorded.

Each participant received the supplement and exercise intervention, either the actual intervention or placebo intervention, and underwent interventions for 8 weeks. After the 8th

week, the post-intervention tests were performed, the same as the first visit for baseline measurement.

2.3.1 Study Design

This study was double blinded, randomized, and placebo-controlled. Two interventions were concurrently introduced to participants; flax lignan supplement intervention and exercise intervention. Four experimental arms were created in a different combination of supplement and exercise training. Subjects were randomized to one of the following four groups by a clinical trials pharmacist in the Royal University Hospital: Flexibility exercise (exercise placebo) + whey protein (supplement placebo), walking exercise + whey protein, flexibility exercise + flax lignan complex, or walking exercise + flax lignan complex. Researchers and participants were blinded from what supplement group each participant was assigned. Due to different appearance between flax lignan complex and whey protein, the two supplements were dispensed by clinical trials pharmacist who had no other involvement with the study.

2.3.2 24h ambulatory blood pressure monitor

Participants were asked to do 24h ambulatory blood pressure monitoring at baseline and after the 8th week. The Oscar 2™ system from SunTech Medical® was used to monitor blood pressure for 24 hours. The accuracy of the device was clinically validated to the European Society of Hypertension (ESH) International protocol, British Hypertension Society (BHS) (A/A), and American National Standards Institute (ANSI)/Association for the Advancement of Medical Instrumentation (AAMI) (SP10). The device was set to measure blood pressure periodically; every 20 minutes during awake time and every 45 minutes during sleeping time. Maximum pressure was set to 180 mmHg. Participants were instructed to wear the cuff for 24 hours. Whenever the device started to measure blood pressure,

subjects were asked to stay still and relax from what they were doing.

2.3.3 Supplementation

The participants consumed the supplement orally in powder form during the 8-week intervention. 800 mg of flax lignan complex (BeneFlax) or 600 mg of whey protein was packaged into a small Ziploc bag. Whey protein was selected as placebo because of its similar form (powder). The 800 mg of flax lignan complex contained 300 mg of SDG. Each bag contained 32.9 % SDG, 13.9 % cinnamic acids, 11.8 % protein, 10.0 % 3-hydroxy-3-methyl glutaric acid, 3.5 % fat, 3.3 % moisture, and 1.0 % ash (Archer Daniels Midland Co., Decatur, IL). Whey protein was from Natural Factors, and each bag of whey protein contained 0.05g of total fat, 0.02g of total carbohydrate, 0.4g of protein and 0.6g of whey protein concentrate (microfiltered and non-denatured). Participants were asked to consume 2 bags per day; therefore, the participants in the flax group consumed about 600 mg of SDG per day. The dose of flax lignan complex was based on a previous human study from Zhang et al. (2007), showing that 600 mg of SDG was sufficient to improve blood cholesterol and glucose level in hypercholesterolemic subjects. A data log book was given to monitor the study compliance.

2.3.4 Exercise protocol

The subjects were randomly assigned to either a walking group or flexibility training group. The flexibility program consisted of the following movements: stretching of lateral neck, inferior and posterior shoulder, shoulder protraction and extension, stretching of gastrocnemius, quadriceps, shoulder, and back, hamstring, groin, hips, and thigh. The walking program was based on the previous study by Cornish et al. (2009). In brief, the participants were asked to do 30-60 minutes walking per day. The intensity was 50 – 65 % of predicted maximal heart rate ($220 - \text{age}$). Subjects were instructed to count the number of

beats in 15 seconds using their radial or carotid pulse. They were instructed to multiply their beats in 15 seconds by 4 to get beats per minute. Subjects were instructed to count their pulse approximately 10 minutes into their exercise and towards the end of their exercise session. They were instructed to increase or decrease the walking speed if their pulse was lower or higher than target heart rate. A data log book was given to monitor the study compliance. Each exercise intervention was done at least 5 times a week.

2.3.5 Blood tests

Blood tests were done at the baseline and after the intervention. An overnight fasting blood sample was collected by a skilled phlebotomist in the morning. The samples were centrifuged at 4000 rpm for 7 minutes. The temperature of the centrifuge was set to 4 Celsius degrees. The plasma aliquot was separated, and the plasma samples were sent to the chemistry lab in the Royal University Hospital, the University of Saskatchewan for analysis. Blood glucose, blood lipids (triacylglyceride (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and total cholesterol (TC)), and C-reactive protein (CRP) were measured from a blood plasma sample. An LX20 Beckman Coulter analyzer (Beckman Coulter Canada Inc., Mississauga, Ont.) was used for analysis of glucose, TG, TC, HDL, and CRP. LDL concentration was calculated using the Friedwald formula.

2.3.6 Food frequency questionnaire

A food frequency questionnaire package was given at baseline and after the intervention to determine the dietary pattern of each participant before and during the interventions. Each time, participants were asked to recall the dietary history during last 2 months. A picture guide was given to estimate the serving portion. The questionnaire packages were sent to NutritionQuest (Berkeley, CA) for analysis.

2.3.7 PASE-Activity Scale

The PASE activity scale developed by New England Research Institute, Inc. in 1991 was used to determine the activity level before and during the interventions. Participants were asked to recall the last 7 days of activity level except the exercise intervention. The questionnaire asked the frequency of physical activities at different intensities (mild, moderate, and vigorous).

2.3.8 Data Analysis

IBM SPSS Statistics (Version 24) was used for the data analysis. The supplement intervention and exercise intervention were analyzed with between-within ANOVA (intervention x time; 2 x 2) with a $p < .05$ as the acceptable level for significance. Although the participants were to be assigned to each group randomly and evenly, there was an imbalance between each experimental arm. One of 4 groups had only 2 subjects in the block, and this led us to analyze the two interventions separately. Therefore, even though two interventions were given concurrently, we did not consider the interaction between the supplement intervention and exercise intervention. Out of 25 participants, 13 subjects were assigned to Flax Lignan Complex (FLC) group and 12 subjects were assigned to the placebo group. Also, 11 subjects were assigned to the walking exercise group, and 14 subjects were assigned to placebo exercise group. For ambulatory blood pressure data analysis, daytime was defined from 9 am to 10 pm and nighttime was defined from 12 am to 6 am.

2.4 Results

2.4.1 Compliance rate

A summary of compliance rate of each group is presented in Table 2.1 and figure 2.1. Each intervention was completed with a high compliance rate. Stretching exercise had over

100% of compliance. The frequency of the exercise interventions was 5 times a week, but some participants performed exercise more than that.

Table 2.1 Compliance rate of each intervention. Each supplement was to be ingested 2 times per day, 7 days/week. Each exercise was performed 5 days/week. Mean \pm SD.

	Whey protein	FLC	Stretching exercise	Walking exercise
Compliance (%)	88.2 \pm 18.3	97.6 \pm 3.7	108.6 \pm 26.6	98.8 \pm 23.0

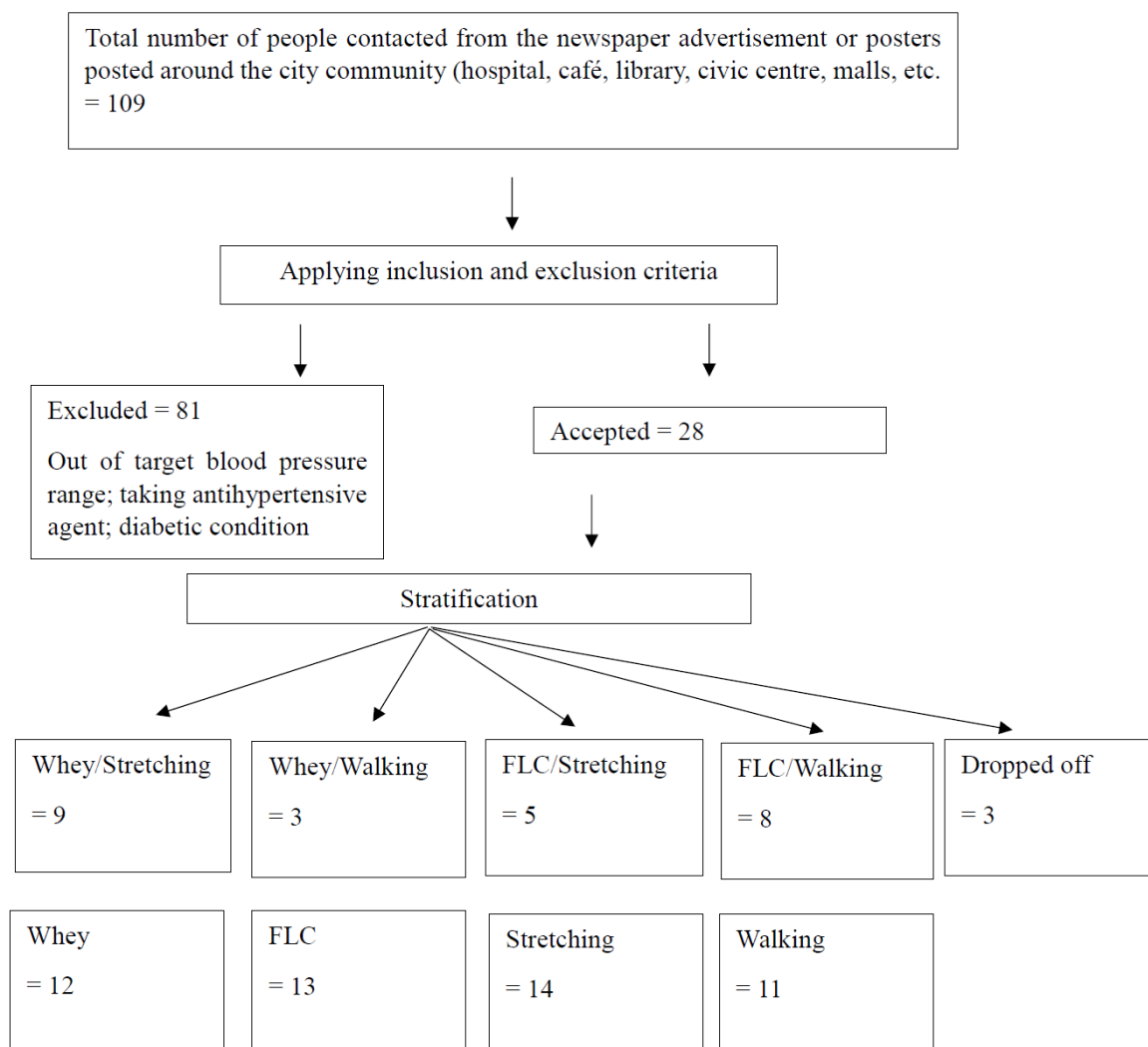


Figure 2.1 Schematic flow diagram of the participant recruitment, exclusion, and stratification into the experimental arms.

2.4.2 Demographic change

Up to 109 individuals contacted the lab for the study, and 82 people were not able to participate in the study because of meeting the exclusion criteria (Figure 2.1). In total, 25 subjects successfully completed the interventions. Sex distribution is summarized in table 2.2. The demographics of the participants in each intervention are described in tables 2.3 and 2.4. There was no significant difference between groups at baseline. Participants showed high compliance rate for both supplement and exercise interventions, with greater than 88% compliance.

After 8 weeks supplement intervention, there was no significant difference in height and weight change within as well as between groups. For waist circumference, there was a significant interaction between exercise group and time ($p = 0.014$). The waist circumference showed significant decreased after the walking exercise intervention, from 110.1 cm to 103.2 cm ($p < .05$).

Table 2.2. Sex distribution in each intervention group.

	Whey protein	FLC	Stretching	Walking
Female	7	8	10	5
Male	5	5	4	6
Total	12	13	14	11

Table 2.3. Pre- and Post- intervention demographics of participants with supplementary interventions. Mean \pm SD

	Whey		Flax Lignan Complex (FLC)		<i>p</i> – value (group x time)
	Pre	Post	Pre	Post	
Age (years)	61.42 ±1.8		61.5 ±2.4		
Height (cm)	165 ±9.6		167.5 ±15.6		
Weight (kg)	81.9 ±21.7	82.4 ±23.5	90.2 ±16.8	90.5 ±17.8	0.895
Waist circumference (cm)	104.5 ±20.6	101.0 ±17.7	106.3 ±12.2	104.4 ±11.5	0.608

Table 2.4 Pre- and Post- intervention demographics of participants with exercise interventions. Mean ± SD. * significant difference between pre and post

	Stretching		Walking		<i>p</i> – value (group x time)
	Pre	Post	Pre	Post	
Age	59.7 ±1.3		63.7 ±2.9		
Height	165.4 ±13.5		167.4 ±12.2		
Weight (kg)	84.6 ±21.8	85.1 ±13.1	88.1 ±16.7	88.4 ±18.2	0.922
Waist circumference	102.1 ±16.5	102.3 ±16.8	110.1 ±16.3	103.2 ±18.2*	0.014

(cm)

2.4.3 Body composition

There was no significant change in DXA measurements after the interventions. There was no significant group difference (Table 2.5).

Table 2.5 Body composition measurement from DXA scanning before and after the supplement and exercise interventions. LBM, lean body mass

	Whey		FLC		<i>p</i> – value (group x time)
	Pre	Post	Pre	Post	
%fat (%)	33.48 ±7.73	32.50 ±7.66	35.10 ±9.74	34.95 ±9.95	0.398
Lean Body					
Mass (LBM) (kg)	51.90±13.00	52.44 ±12.66	56.09 ±14.55	56.20 ±14.73	0.412
Whole body					
fat mass (kg)	27.98 ±11.50	27.34 ±12.62	31.36 ±9.50	31.17 ±9.66	0.671
Trunk fat					
mass (kg)	14.50 ±6.05	13.60 ±7.00	15.83 ±4.90	15.68 ±5.07	0.510
	Stretching		Walking		
	Pre	Post	Pre	Post	

%fat (%)	34.98 ±8.56	34.86 ±9.25	33.32 ±9.12	32.14 ±8.28	0.283
LBM (g)	52.58 ±14.62	52.61 ±14.45	55.98 ±12.68	56.71 ±12.57	0.412
Whole body fat mass (kg)	29.84 ±11.04	30.01 ±12.43	29.43 ±10.17	28.20 ±9.66	0.178
Trunk fat mass (kg)	14.92 ±5.58	14.98 ±6.82	15.51 ±5.48	14.16 ±5.15	0.218

2.4.4 24h ambulatory blood pressure

FLC supplementation

After 8 weeks of the supplement intervention, there was no significant change in 24h ambulatory blood pressure (systolic/diastolic), heart rate, mean arterial pressure, pulse pressure in either FLC or placebo group and no significant between-group difference was found (Table 2.6).

Table 2.6 24-h ambulatory blood pressure (mmHg), heart rate (BPM), mean arterial pressure (mmHg), and pulse pressure before and after the supplementary intervention. Mean ± SD. Flax Lignan Complex (FLC); blood pressure (BP)

	Whey		FLC		<i>p – value</i> <i>group x time</i>
	Pre	Post	Pre	Post	
Systolic BP	138.8 ±7.3	138.5 ±10.9	143.8 ±7.9	143.5 ±9.6	0.993

(mmHg)					
Diastolic BP	80.6 ±7.6	80.9 ±8.8	80.2 ±5.6	80.2 ±7.5	0.860
(mmHg)					
Heart rate	71.8 ±10.3	72.7 ±8.4	71.7 ±10.2	72.1 ±9.3	0.829
(BPM)					
Mean arterial					
pressure	100.0 ±6.9	100.1 ±8.6	101.2 ±4.6	101.2 ±7.1	0.969
(mmHg)					
Pulse pressure	58.3 ±6.4	57.7 ±8.0	63.5 ±9.6	63.2 ±9.2	0.928
(mmHg)					

Exercise intervention

After 8 weeks walking exercise, there was no significant difference in 24h ambulatory blood pressure in walking and placebo groups, and no significant between-group difference was found (Table 2.7).

Table 2.7 24-h ambulatory blood pressure, heart rate, mean arterial pressure, and pulse pressure before and after the exercise intervention. Statistical analysis was performed with a confidence interval of 95%. Mean ± SD. Blood pressure (BP).

Stretching		Walking		<i>p</i> - value
Pre	Post	Pre	Post	<i>group x time</i>

Systolic BP (mmHg)	140.3 ±8.0	138.4 ±10.3	142.8 ±7.9	144.6 ±9.7	0.240
Diastolic BP (mmHg)	80.9± 6.1	79.6 ±8.2	79.8 ±7.2	81.8 ±7.8	0.073
Heart rate (BPM)	71.3 ±4.1	71.9 ±6.3	72.4 ±14.8	72.9 ±11.4	0.963
Mean arterial pressure (mmHg)	100.6 ±6.1	99.1 ±8.1	100.6 ±5.5	102.6 ±7.1	0.096
Pulse pressure (mmHg)	59.5 ±6.3	58.9 ±8.0	62.9± 10.8	62.6 ±10.0	0.925

2.4.5 Daytime and nighttime ambulatory hourly blood pressure

From supplementation interventions, there was a significant interaction between group and time for nighttime heartrate ($p = 0.019$) favoring to FLC group (Table 2.8). From exercise interventions, there were significant interactions between group and time in nighttime diastolic blood pressure ($p = 0.042$) and nighttime mean arterial pressure ($p = 0.036$) favouring to the stretching exercise group (Table 2.9).

When the daytime and nighttime ambulatory blood pressure data were analyzed in hourly, there was no significant difference in hourly blood pressure before and after the supplementary or exercise intervention (Figure 2.2; Figure 2.3; Figure 2.4; Figure 2.5; Figure 2.6; Figure 2.7; Figure 2.8; Figure 2.9).

Table 2.8 Daytime and nighttime ambulatory blood pressure, heart rate, mean arterial pressure, and pulse pressure before and after the supplementation intervention. Statistical analysis was performed with a confidence interval of 95%. Mean \pm SD. Blood pressure (BP).

	Whey		FLC		<i>p</i> – value
	Pre	Post	Pre	Post	<i>group x time</i>
Daytime					
Systolic BP (mmHg)	145.1 \pm 6.4	144.8 \pm 11.5	148.0 \pm 7.5	145.6 \pm 7.1	0.569
Daytime					
Diastolic BP (mmHg)	85.2 \pm 7.0	85.7 \pm 8.3	84.7 \pm 7.1	83.3 \pm 8.2	0.429
Nighttime					
Systolic BP (mmHg)	120.8 \pm 11.0	119.2 \pm 12.4	125.8 \pm 12.5	126.4 \pm 11.4	0.539
Nighttime					
Diastolic BP (mmHg)	66.7 \pm 9.6	67.3 \pm 11.6	67.7 \pm 6.3	68.2 \pm 11.1	0.978
Daytime Heart rate (BPM)	74.8 \pm 11.5	74.8 \pm 9.4	74.8 \pm 10.2	75.4 \pm 8.7	0.635
Nighttime Heart rate (BPM)	63.1 \pm 6.4	66.0 \pm 5.5	67.3 \pm 9.1	64.5 \pm 7.0	0.019*

Daytime Mean					
arterial pressure	105.2±6.0	105.8±5.9	105.1± 8.3	103.9±7.2	0.513
(mmHg)					
Nighttime Mean					
arterial pressure	84.8±9.7	84.4 ±11.3	87.0±7.4	87.5±10.4	0.785
(mmHg)					
Daytime Pulse					
pressure	59.8±6.9	59.6±8.8	63.5±8.4	62.3±7.5	0.729
(mmHg)					
Nighttime Pulse					
pressure	53.8±6.4	52.0±7.8	58.1±10.4	58.6±8.4	0.406
(mmHg)					

Table 2.9 Daytime and nighttime ambulatory blood pressure, heart rate, mean arterial pressure, and pulse pressure before and after the exercise intervention. Statistical analysis was performed with a confidence interval of 95%. Mean ± SD. Blood pressure (BP).

	Stretching		Walking		<i>p – value</i> <i>group x time</i>
	Pre	Post	Pre	Post	
Daytime Systolic BP	146.7± 7.5	144.4±10.4	146.3±6.5	146.4±8.0	0.524

(mmHg)					
Daytime					
Diastolic BP	85.6± 5.6	84.1±7.9	84.0±8.7	84.9±8.9	0.342
(mmHg)					
Nighttime					
Systolic BP	122.5±12.3	119.1± 3.0	124.3±11.6	127.9±9.4	0.052
(mmHg)					
Nighttime					
Diastolic BP	67.6±8.6	65.7±11.2	66.5±7.4	70.5±10.9	0.042
(mmHg)					
Daytime Heart rate (BPM)	73.5±4.7	74.2±7.5	76.7±15.9	77.1±10.3	0.900
Nighttime Heart rate (BPM)	65.3±4.7	65.0±4.4	65.1±11.4	65.6±8.4	0.766
Daytime Mean					
arterial pressure	106.0±5.5	103.9±7.7	104.7±6.5	105.3±7.9	0.321
(mmHg)					
Nighttime Mean					
arterial pressure	86.0±9.4	83.5±11.4	85.7±7.6	89.4±9.3	0.036
(mmHg)					
Daytime Pulse pressure	62.2±6.6	60.6±8.6	62.3±9.5	61.5±7.7	0.953

(mmHg)

Nighttime Pulse

pressure 54.7 ± 7.5 53.6 ± 7.0 57.7 ± 10.3 57.7 ± 10.3 0.690

(mmHg)

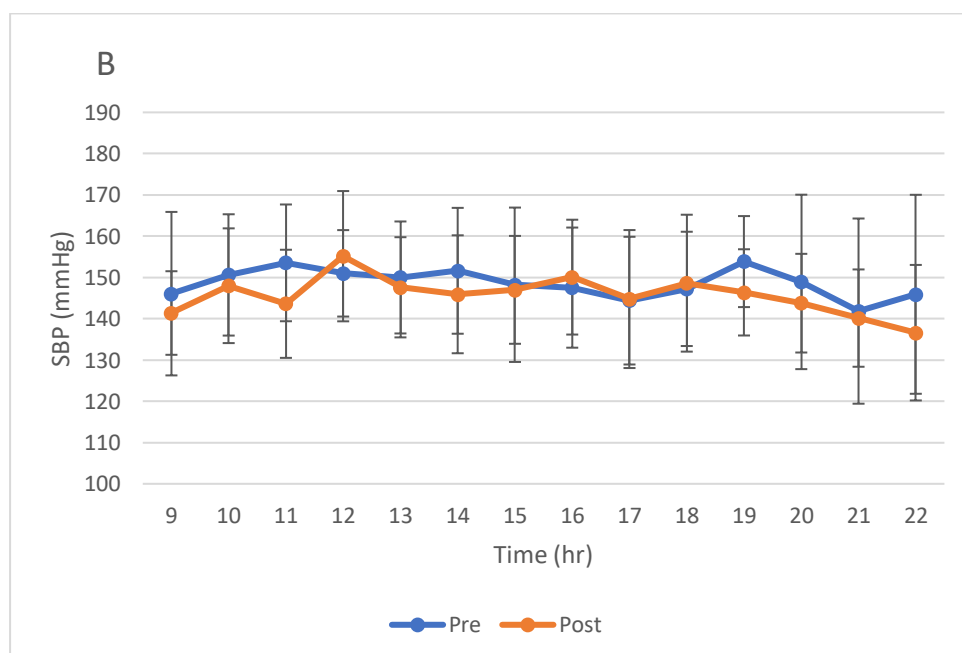
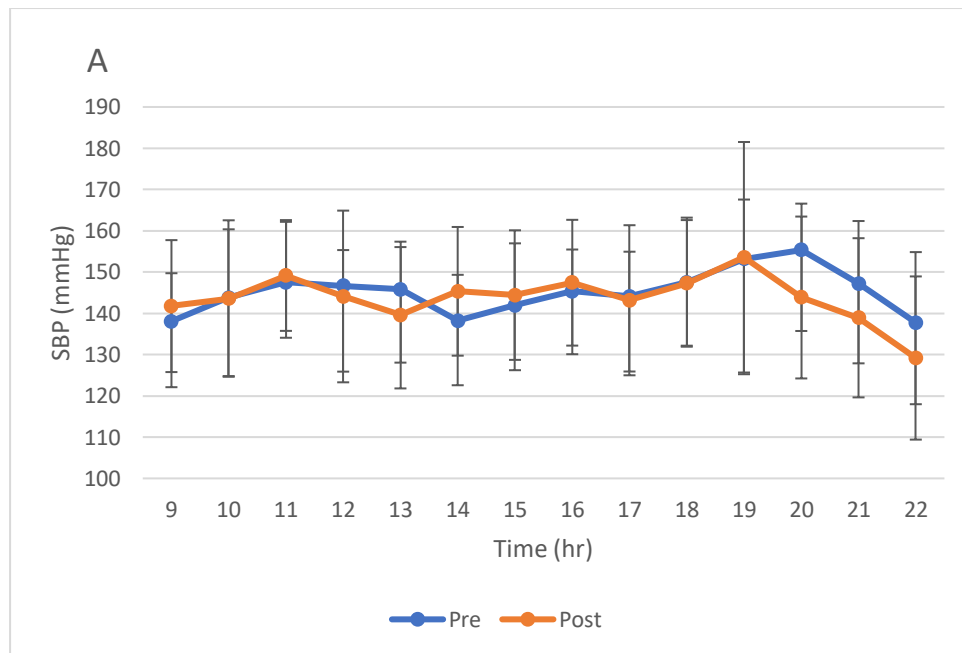


Figure 2.2 Daytime ambulatory systolic blood pressure before and after the whey protein supplementation (A) and FLC supplementation (B). Mean \pm SD.

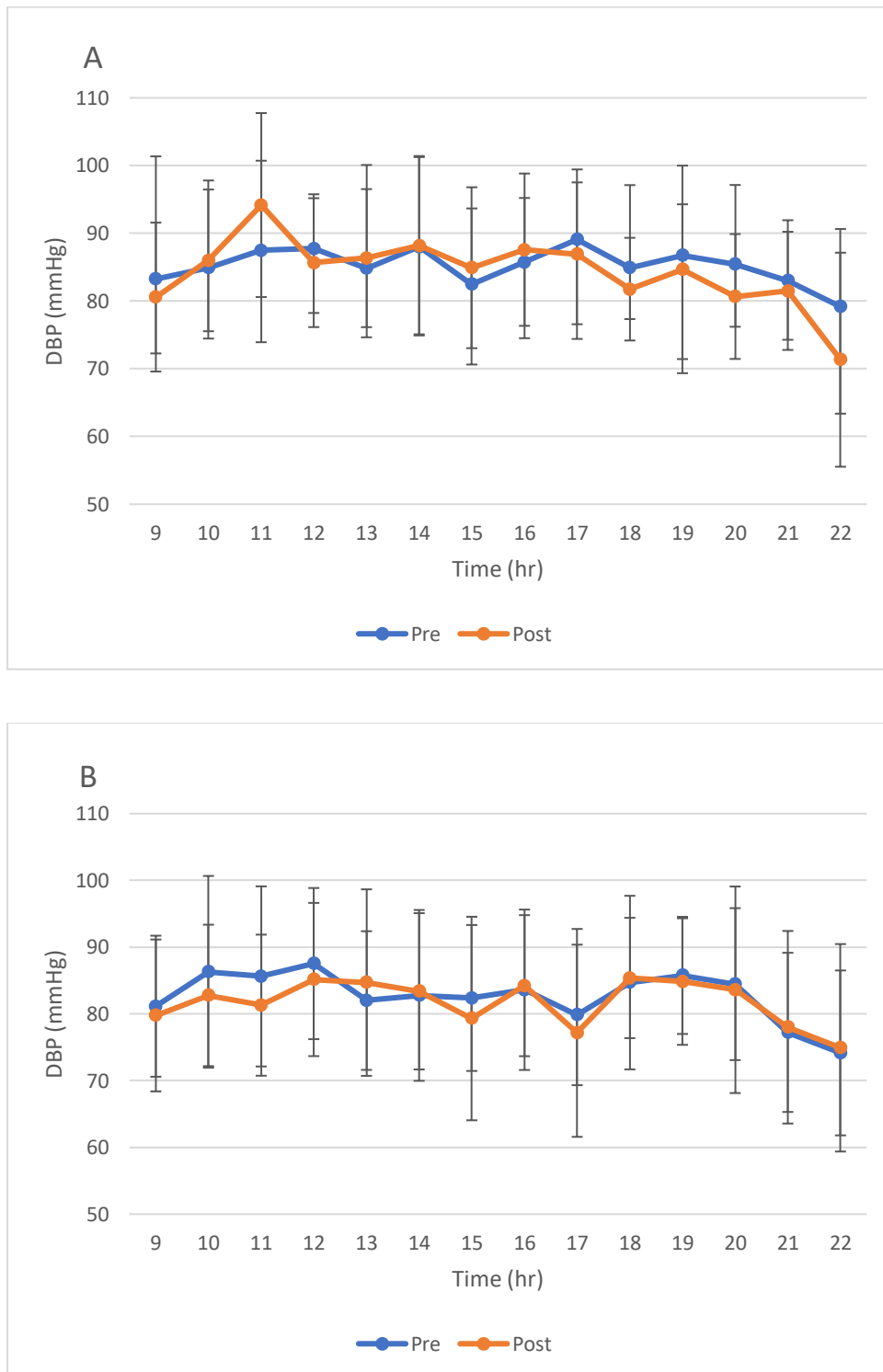


Figure 2.3 Daytime ambulatory diastolic blood pressure before and after whey protein

supplementation (A) and FLC supplementation (B). Mean \pm SD.

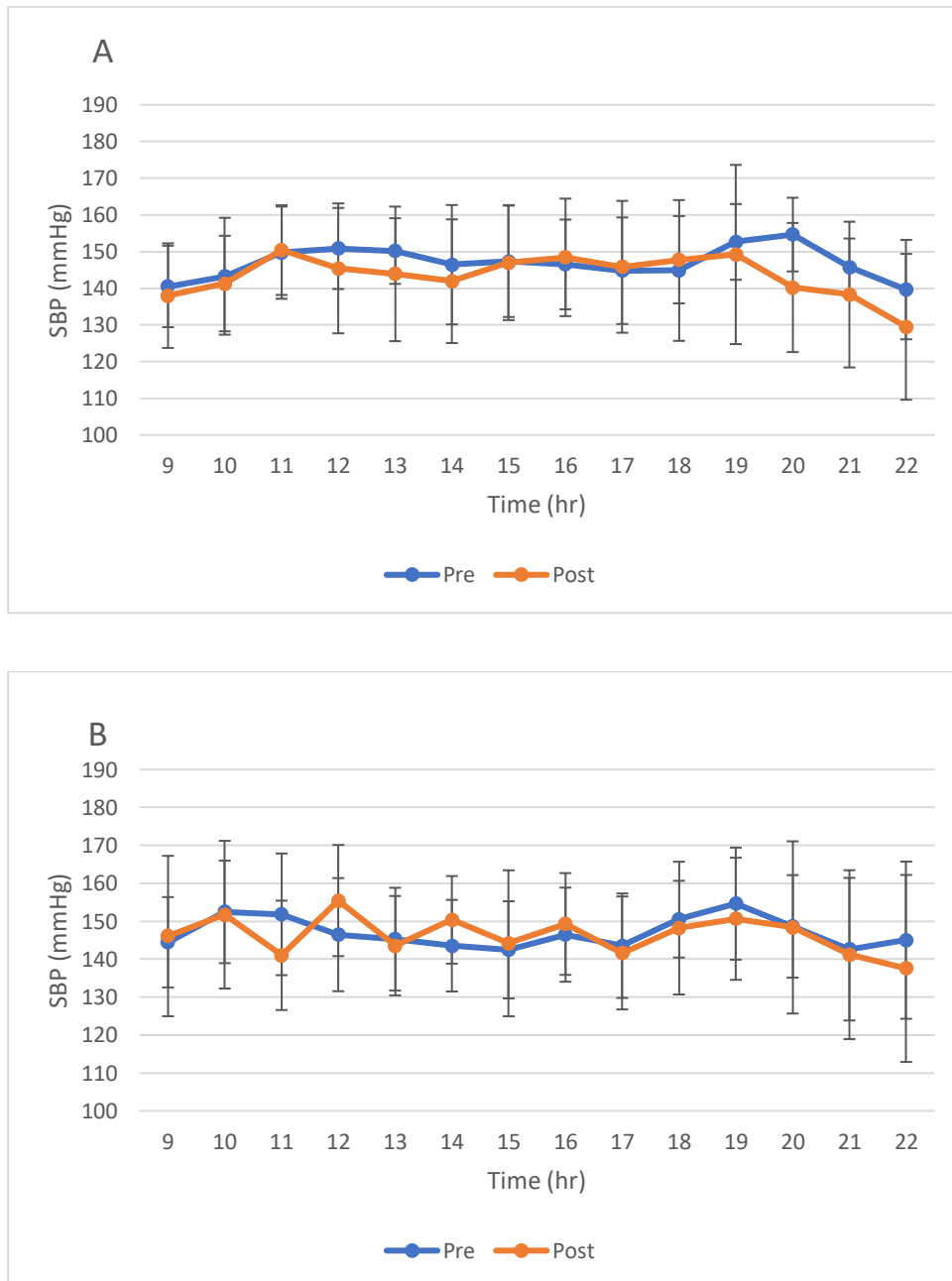


Figure 2.4 Daytime ambulatory systolic blood pressure before and after the stretching exercise (A) and walking exercise (B). Mean \pm SD.

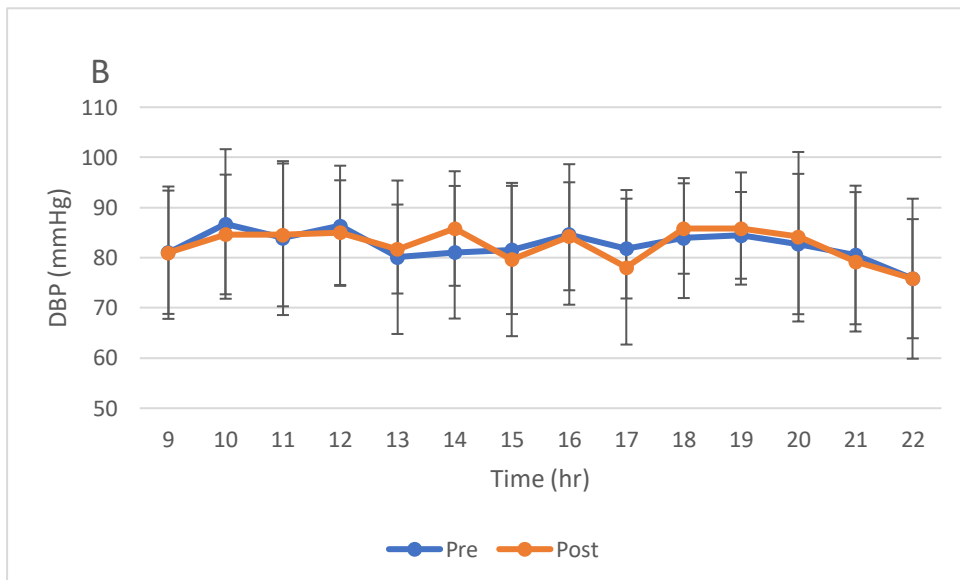
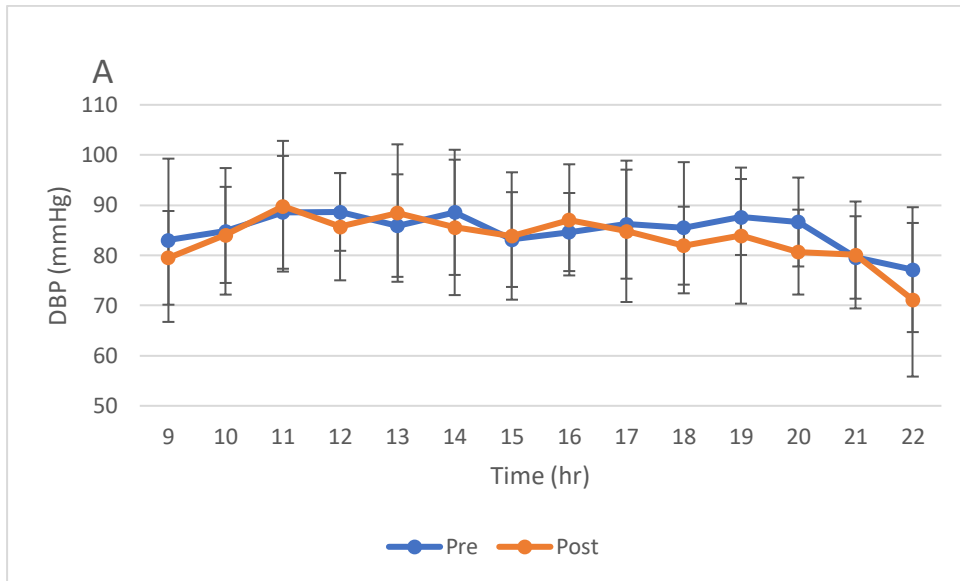


Figure 2.5 Daytime ambulatory diastolic blood pressure before and after the stretching exercise (A) and walking exercise (B). Mean \pm SD.

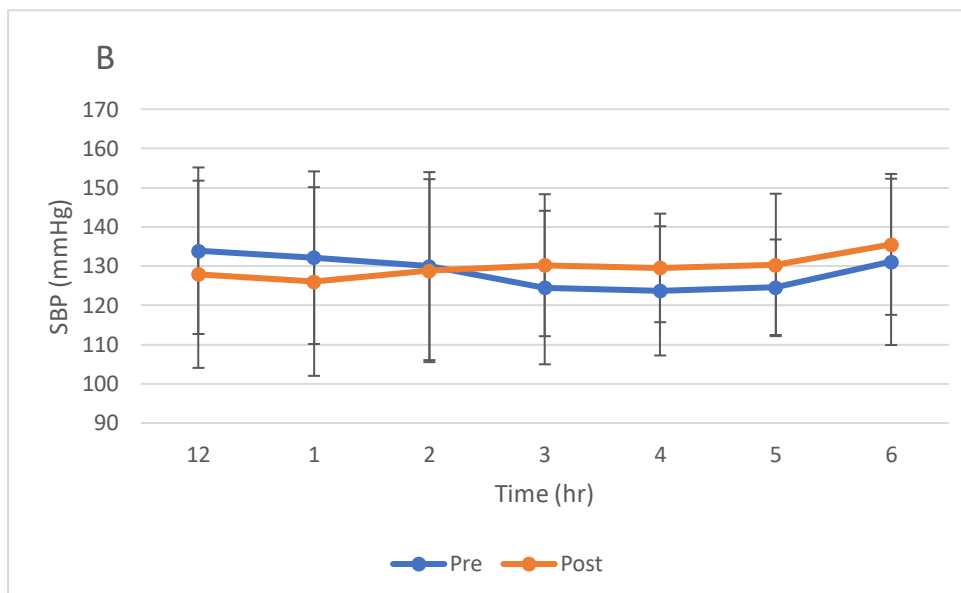
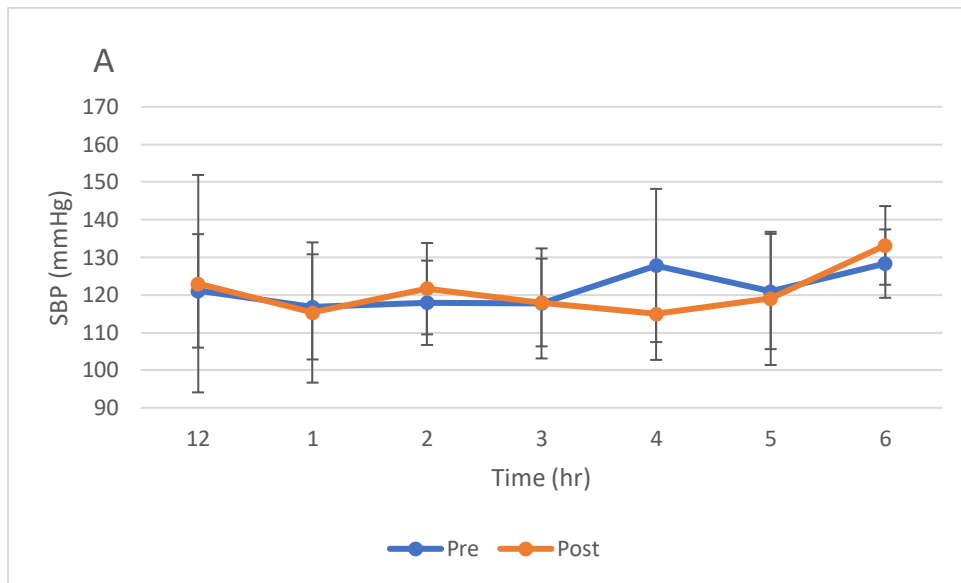


Figure 2.6 Sleep time ambulatory systolic blood pressure before and after the whey protein supplementation (A) and FLC supplementation (B). Mean \pm SD.

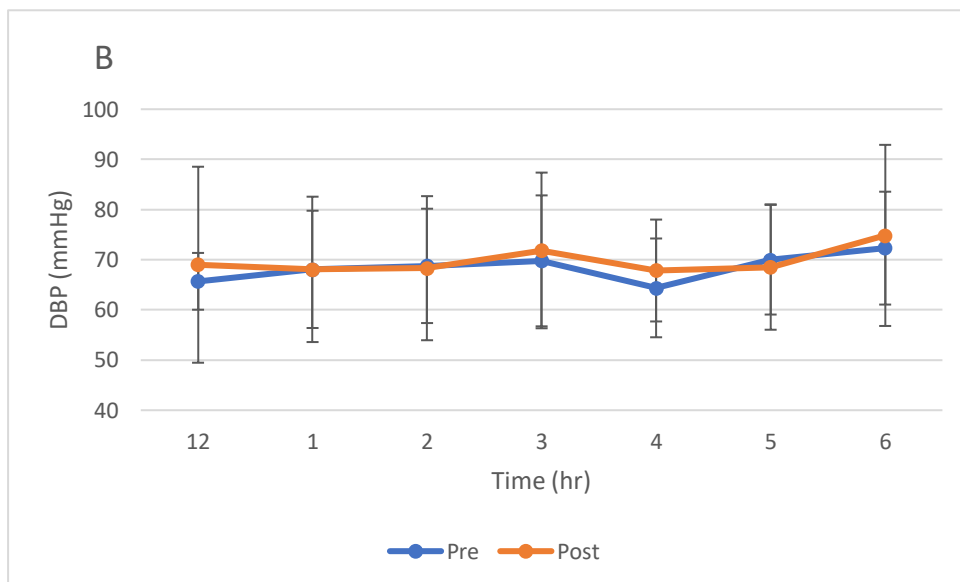
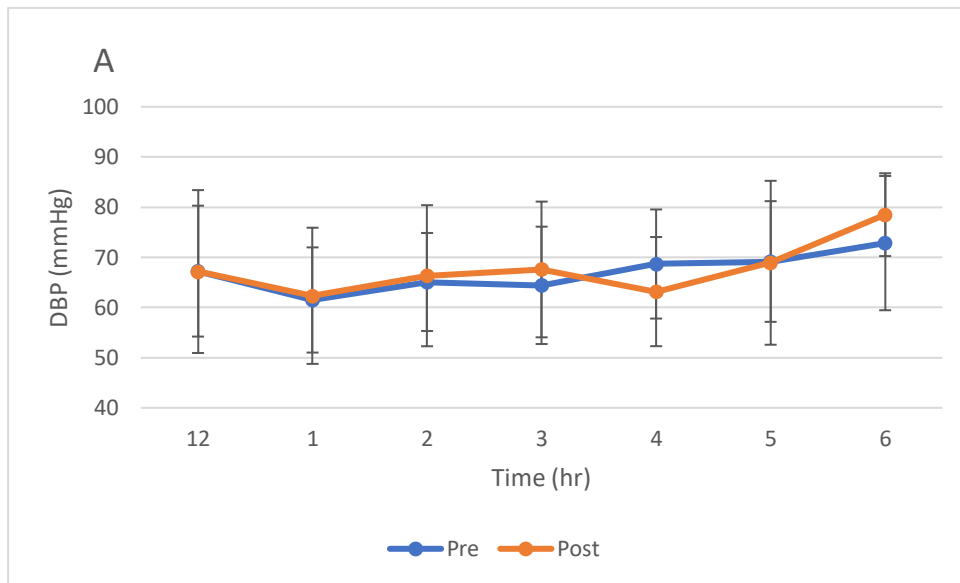


Figure 2.7 Sleep time ambulatory diastolic blood pressure before and after whey protein supplementation (A) and FLC supplementation (B). Mean \pm SD.

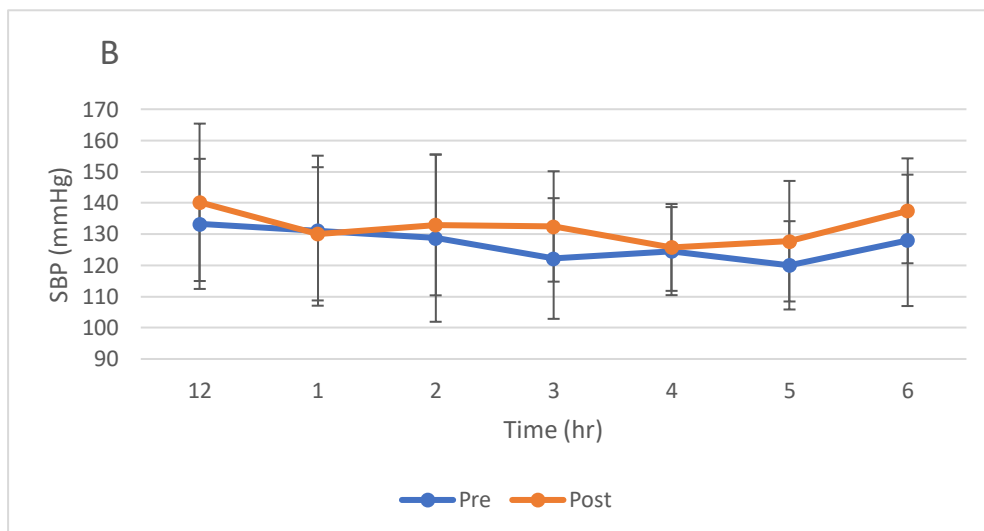
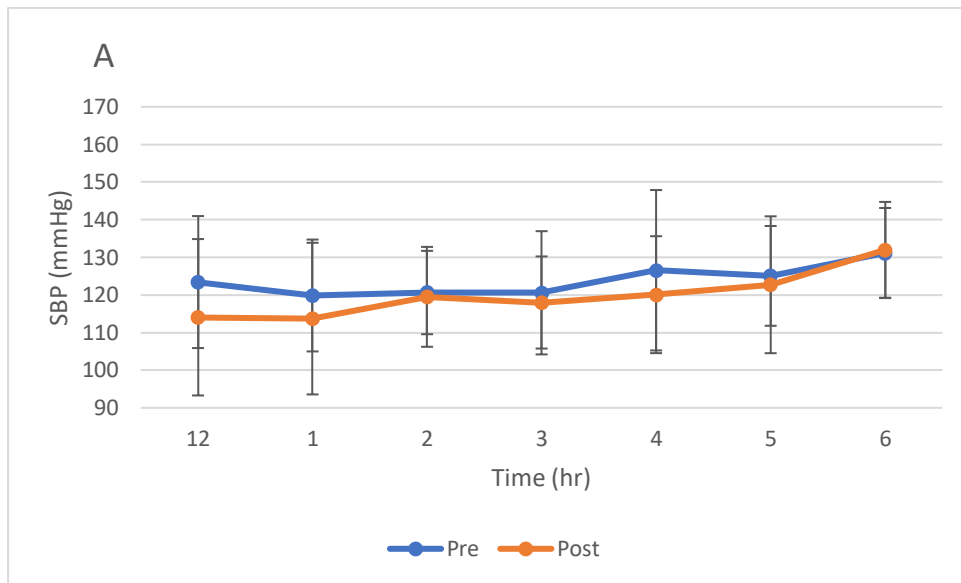


Figure 2.8 Sleep ambulatory systolic blood pressure before and after the stretching exercise (A) and walking exercise (B). Mean \pm SD.

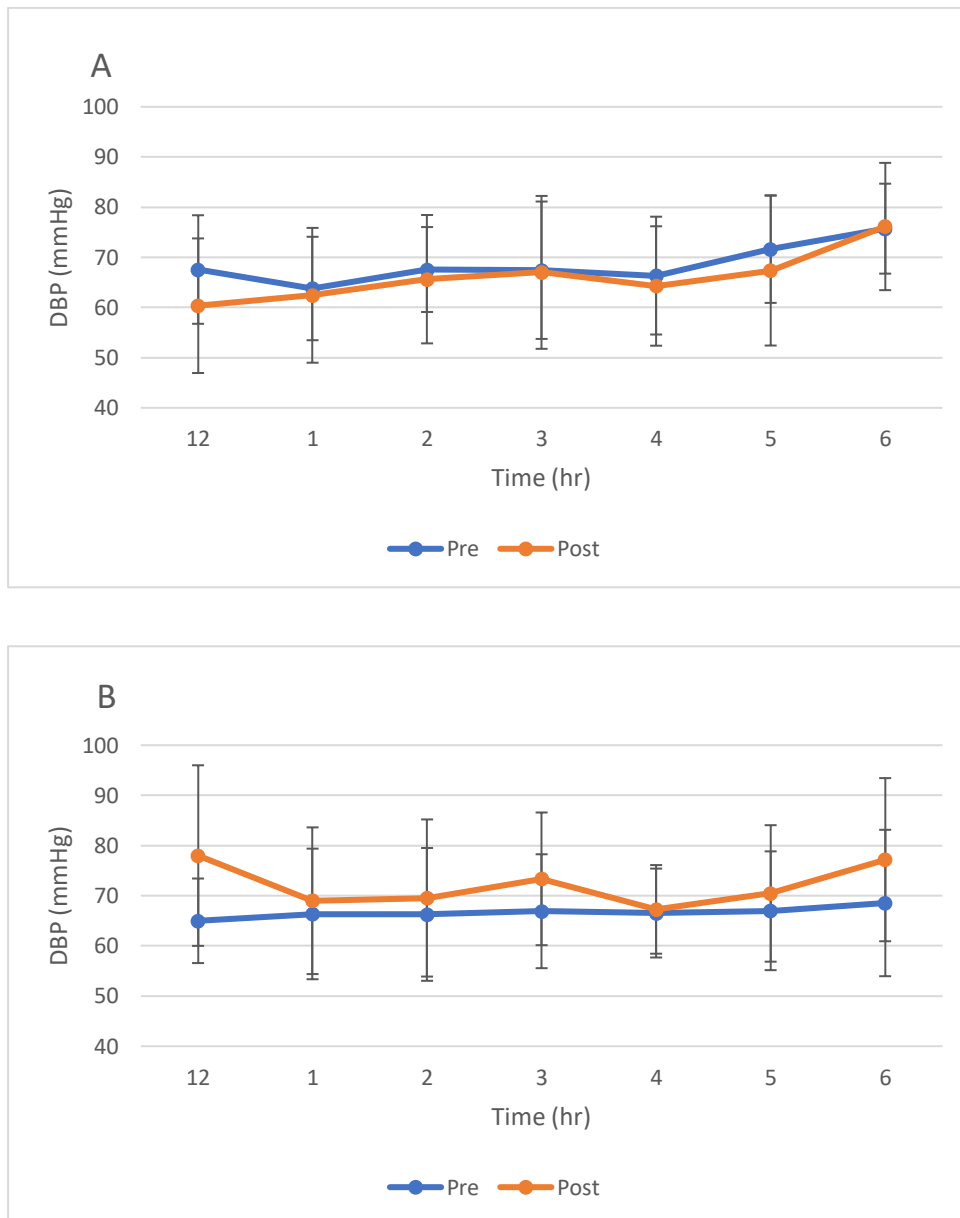


Figure 2.9 Sleep ambulatory diastolic blood pressure before and after the stretching exercise (A) and walking exercise (B). Mean \pm SD.

2.4.6 Blood analysis (blood glucose, total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), tri-acyl glyceride (TAG), C-reactive protein

FLC supplementation intervention

After 8 weeks of supplement intervention, there was no significant change in cholesterol level. There was a significant interaction between supplement and time in TAG, HDL level

and cholesterol:HDL ratio, in favour of the placebo group (Table 2.10).

Table 2.10 The blood level of cholesterol, TG, high-density lipoprotein, low-density lipoprotein, glucose, and c-reactive protein before and after the supplementary intervention. Mean \pm SD. TG, triacylglycerides; HDL, high-density lipoprotein; LDL, low-density lipoprotein; FLC, flax lignan complex. Reference value for glucose was 3.6 – 6.0 mmol/L; Reference value for cholesterol was 4.20 – 5.20 mmol/L; Reference value for TG was 0.60 – 2.30 mmol/L; Reference value for HDL was 0.90 – 2.40 mmol/L; Reference value for LDL was 2.20 – 3.40 mmol/L; Reference value for c-reactive protein was 0.0 – 7.0 mg/L.

	Whey		FLC		<i>p</i> – value
	Pre	Post	Pre	Post	<i>group x time</i>
Cholesterol (mmol/L)	5.60 \pm 0.85	5.43 \pm 0.82	5.93 \pm 0.87	6.02 \pm 0.83	0.222
TG (mmol/L)	1.66 \pm 0.75	1.21 \pm 0.44	1.48 \pm 0.68	1.68 \pm 0.74	0.008
HDL (mmol/L)	1.32 \pm 0.32	1.44 \pm 0.37	1.41 \pm 0.60	1.36 \pm 0.61	0.014
LDL (mmol/L)	3.50 \pm 0.75	3.44 \pm 0.81	3.81 \pm 0.82	3.82 \pm 0.67	0.660
Cholesterol:HDL	4.26 \pm 0.90	3.93 \pm 1.16	4.76 \pm 1.77	4.98 \pm 1.76	0.024
Glucose (mmol/L)	5.55 \pm 0.61	5.49 \pm 0.51	5.90 \pm 1.23	5.90 \pm 1.13	0.752
C-reactive protein (mg/L)	2.47 \pm 2.52	2.33 \pm 2.65	4.10 \pm 3.16	3.47 \pm 2.19	0.516

Walking exercise Intervention

There was significant interaction in total cholesterol level between exercise type and time. Interestingly, the change was a favour to the stretching exercise group (Table 2.11).

Table 2.11 The blood level of cholesterol, TG, HDL, LDL, glucose, and c-reactive protein before and after the exercise intervention. Statistical analysis was performed with a confidence interval of 95%. Mean \pm SD. TG, triacylglycerides; HDL, high-density lipoprotein; LDL, low-density lipoprotein; FLC, flax lignan complex. Reference value for glucose was 3.6 – 6.0 mmol/L; Reference value for cholesterol was 4.20 – 5.20 mmol/L; Reference value for TG was 0.60 – 2.30 mmol/L; Reference value for HDL was 0.90 – 2.40 mmol/L; Reference value for LDL was 2.20 – 3.40 mmol/L.

	Stretching		Walking		<i>p</i> – value
	Pre	Post	Pre	Post	<i>group x time</i>
Cholesterol (mmol/L)	5.58 \pm 0.93	5.43 \pm 0.75	5.93 \pm 0.82	6.02 \pm 0.96	0.049
TAG (mmol/L)	1.66 \pm 0.71	1.21 \pm 0.49	1.48 \pm 0.72	1.68 \pm 0.71	0.191
HDL (mmol/L)	1.32 \pm 0.36	1.44 \pm 0.41	1.41 \pm 0.59	1.36 \pm 0.57	0.362
LDL (mmol/L)	3.60 \pm 0.90	3.44 \pm 0.69	3.81 \pm 0.65	3.82 \pm 0.80	0.155
Cholesterol:HDL	4.00 \pm 1.04	3.83 \pm 1.13	5.29 \pm 1.67	5.51 \pm 1.67	0.117
Glucose (mmol/L)	5.55 \pm 0.57	5.49 \pm 0.53	5.90 \pm 1.35	5.90 \pm 1.17	0.126

C-reactive protein (mg/L)	2.47 ±3.55	2.33 ±3.00	4.05 ±1.74	3.47 ±1.36	0.446
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2.4.7 6 minutes walking test

After 8 weeks of supplementary and exercise intervention, there was no significant change in the distance covered from 6 minutes walking test (Table 2.12).

Table 2.12 Distance covered by participants during 6 minutes walking test. Statistical analysis was performed with confidence level of 95%. Mean ± SD. 6mwt, 6 minutes walking test; FLC, flax lignan complex.

	Whey		FLC		<i>p</i> – value
	Pre	Post	Pre	Post	group x time
6 minute walking test (6mwt) (m)	563.0 ±84.4	577.3 ±90.9	554.7 ±74.5	575.1 ±76.0	0.729

	Stretching		Walking		<i>p</i> – value
	Pre	Post	Pre	Post	group x time
6 mwt (m)	578.9 ±82.6	590.3 ±86.6	530.9 ±64.6	556.4 ±74.7	0.426

2.4.8 FFQ and PASE

There was no significant difference between groups before and after the interventions for dietary or physical activity analyses (Table 2.13).

Table 2.13 Change in PASE score before and after the interventions. Statistical analysis was performed with a confidence interval of 95%. Mean \pm SD. PASE, physical activity for older adults; FLC, flax lignan complex.

	Whey		FLC		<i>p</i> – value
	Pre	Post	Pre	Post	group x time
PASE score	163.4 \pm 42.1	178.4 \pm 71.1	151.3 \pm 60.4	136.1 \pm 76.8	0.221

	Stretching		Walking		<i>p</i> – value
	Pre	Post	Pre	Post	group x time
PASE score	152.6 \pm 52.9	167.6 \pm 75.9	163.1 \pm 51.2	144.9 \pm 76.9	0.178

There was no significant difference or change in daily carbohydrates (CHO), fat, and protein consumption between the groups, as well as before and after the interventions (Table 2.14).

Table 2.14 Change in macronutrient distribution before and after the intervention. Statistical analysis was performed with a confidence interval of 95%. Mean \pm SD

	Whey supplementation		FLC supplementation		<i>p</i> – value
	Pre	Post	Pre	Post	group x time
CHO					
ingested (g/day)	188.10 \pm 69.97	174.35 \pm 71.42	189.83 \pm 76.18	173.87 \pm 52.60	0.889
Fat	84.46 \pm 34.23	79.83 \pm 34.97	90.46 \pm 39.79	76.97 \pm 34.46	0.388

ingested

(g/day)

Protein

ingested	69.47 ±25.98	68.24 ±27.61	76.68 ±30.05	73.16 ±18.75	0.770
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(g/day)

		Stretching exercise		Walking exercise		
		Pre	Post	Pre	Post	
CHO						
ingested	182.29 ±73.67	163.67 ±56.70	197.73 ±71.75	187.65 ±66.35	0.589	
(g/day)						
Fat						
ingested	81.52 ±35.82	72.14 ±28.67	95.47 ±37.77	86.39 ±39.90	0.977	
(g/day)						
Protein						
ingested	75.30 ±28.12	70.65 ±24.32	70.55 ±28.61	71.01 ±22.99	0.515	
(g/day)						

2.4.10 Power observed

Due to the low number of participants, observed power of main outcomes was very low (Table 2.15; Table 2.16). Therefore, results of this study need to be interpreted with caution.

Table 2.15 Observed power of effect from 8-week supplementation on ambulatory blood pressure (24h, daytime, and nighttime). $\alpha = 0.05$

	Supplement (whey vs FLC)	Time (Pre vs Post)	Supplement x time
24h SBP	0.306	0.054	0.050
24h DBP	0.054	0.053	0.053
Daytime SBP	0.095	0.104	0.086
Daytime DBP	0.077	0.066	0.120
Nighttime SBP	0.258	0.057	0.092
Nighttime DBP	0.057	0.064	0.050

Table 2.16 Observed power of 8-week exercise on ambulatory blood pressure (24h, daytime, and nighttime). $\alpha = 0.05$

	Exercise (stretching vs walking)	Time (Pre vs Post)	Exercise x time
24h SBP	0.241	0.050	0.211
24h DBP	0.055	0.068	0.435
Daytime SBP	0.058	0.088	0.095
Daytime DBP	0.052	0.055	0.153
Nighttime SBP	0.196	0.051	0.503
Nighttime DBP	0.075	0.112	0.543

Chapter 3: Discussion

Flaxseed has been highlighted as a functional food exerting various health beneficial effects. These include protection against cardiovascular risks, improvement in blood glucose and lipid profile, as well as protection against cancers (Calado et al. 2018; Azrad et al. 2013). While the flaxseed is comprised of various ingredients, such as fibre and omega-3 fatty acids, plant lignan, specifically secosolariciresinol diglucoside (SDG), has been closely studied in animal and human subjects to investigate the effect and its mechanisms in the body. Prehypertension and stage I hypertension could be managed by changing lifestyles such as eating habits and regular physical activity, and this study attempted to see the change in blood pressure and other blood parameters after applying regular walking exercise and FLC supplementation. To determine the effect of flax lignan complex (FLC) on blood pressure of older participants with pre-hypertension or stage I hypertension, 25 participants completed the 8 weeks FLC supplementary intervention with walking exercise intervention. As a primary measurement, 24-hour ambulatory blood pressure was measured, and overnight fasting blood samples were analyzed to see the change in glucose and lipid profiles. After the 8-week intervention, there was no significant change in systolic and diastolic ambulatory blood pressure, heart rate, mean arterial pressure, or pulse pressure. Interestingly, there was a significant interaction between time and supplement in HDL and LDL level, favouring the whey protein placebo group. There was also a significant interaction between time and exercise in total cholesterol level favouring the stretching group.

SDG has been studied closely in both humans and animal models, and studies showed the potential to be used as an antihypertensive agent by reducing systolic and/or diastolic blood pressure significantly. However, results from the current study did not agree with the hypotensive effect of SDG supplementation. In a study by Leyva et al. (2013), the blood

pressure of peripheral arterial disease patients was reduced by 10 mmHg in systolic and 7 mmHg in diastolic after 6 months of flaxseed supplementation. When SDG or flaxseed is administrated to healthy individuals, the results were often mixed. In the previous study by Cornish et al. (2009), after 6 months of SDG supplementation and exercise, diastolic blood pressure of healthy male participants was decreased relative to the male placebo group. However, the other targeted healthy menopausal women showed no effect on endothelial function from flax lignan administration for 6 months, thus no significant change in blood pressure (Hallund et al., 2006). Another study targeted healthy older adults (60-80 years) and also showed no change in blood pressure after 24 weeks of FLC supplementation (Alcorn et al., 2017). These three studies used a similar daily dose of SDG (543mg and 600 mg vs. 500 mg) and same duration of intervention time (~6 months) but yielded different results. These differences could be due to the sex difference. Hallund et al. (2006) only included menopausal females, and Cornish et al. (2009) were able to see a hypotensive effect only when males were analyzed. The menopausal women study pointed out that the fiber might be the main component exerting the beneficial effect, while SDG is possibly just assisting (Hallund et al., 2006). Dietary fiber is well known to provide prevention and/or improvement in many metabolic conditions such as obesity, high blood glucose and lipid level (Aleixandre and Miguel, 2008). These conditions are highly associated with hypertension. Although flaxseed is the richest source for the lignan, other food sources, such as rye, wheat, sesame, asparagus, nuts and legumes still contain lignan (Smeds et al., 2007). Those food sources are very commonly consumed, and this might interfere with the detection of differences between placebo and FLC groups since the diet plan was not controlled in this study. It should be noted that there was no significant difference in total enterolactone level with 10g – 30g of daily flaxseed ingestion (Edel et al., 2015). Each gram of flaxseed contains about 9 – 30 mg of lignan precursor, and according to this, 10-30 g of flaxseed can contain about 90 – 900 mg

of lignan (Toure and Xueming, 2010). Other studies using flax lignan showed beneficial effect using similar daily dose (Zhang et al., 2008; Cornish et al., 2009; Hallund et al., 2006). Therefore, the FLC dose (600 mg/day) used in this study could still be sufficient for proper bioavailability and beneficial effect.

Many studies conducted interventions in slightly different settings and targeted different populations, and this led to mixed results in between the studies, including the current study. Many studies included participants already taking one or more anti-hypertensive agents as well as cholesterol-lowering medication. Although there were mixed results in blood pressure change after supplementation of flaxseed or SDG, some studies showed prominent results that the SDG could provide an anti-hypertensive effect, especially when the study targeted a population with hypertension, such as those who had peripheral artery disease (PAD) and/or hyperlipidemia. The FLAX-PAD study raised the possibility that reduction in blood pressure after flaxseed supplementation could be due to the interaction or synergetic effect between the flaxseed and antihypertensive agents (Rodriguez-Leyva et al., 2013). Participants in a study of the cholesterol-lowering effect of flaxseed were using cholesterol-lowering medication at the same time (Edel et al., 2015). Both studies recruited participants with peripheral artery disease, while our study only included otherwise healthy individuals with prehypertension or stage I hypertension. Participants from the current study were not using any antihypertensive agent or diuretics which can alter blood pressure, and we attempted to determine the effect solely from the FLC supplementation. While the current study included individuals with prehypertension and stage I hypertension, according to the seventh report from Joint National Committee (2003), prehypertension does not require drug therapy unless there is a compelling indication such as kidney diseases or diabetes, but stage I hypertension requires initial drug therapy even without compelling indication. Therefore, these two groups

may need to be analyzed separately. In this study, subgroup analyzes could not be achieved due to the low number of participants. A future study with a larger number of participants will be necessary to revisit the results from the current study.

The current study focused on the lignan component of flaxseed, but many of the studies used whole flaxseed, which contains other beneficial components such as ALA and fiber. Previous studies that used milled flaxseed or food containing milled flaxseed showed significant reductions in both systolic and diastolic blood pressure in hypertension patients (Rodriguez-Leyva et al., 2013; Caligiuri et al., 2014). High alpha-linolenic acid content in flaxseed alters the oxylipin level by inhibiting epoxide hydrolase and promoting vasodilation and reduced inflammation (Caligiuri et al., 2014). Although flaxseed is high in beneficial polyunsaturated fatty acids, such as ALA and eicosapentaenoic acid, the enzyme called epoxide hydrolase metabolizes beneficial oxylipin into harmful ones, such as dihydroxyoctadecenoic acid and dihydroxyeicosatrienoic acid, leading to inflammation in endothelial cells and loss of vasodilation (Caligiuri et al., 2014; Schmelzer et al., 2005; Koeners et al., 2011). Specifically, flaxseed supplementation could reduce the activity of epoxide hydrolase, leading to a reduction in epoxide hydrolase-derived oxylipin and reduction in blood pressure (Caligiuri et al., 2014). It is noteworthy that plant lignan is not the only beneficial bioactive component in flaxseed, and a combination of other components of the flaxseed may increase the hypotensive effect via different mechanisms. A meta-analysis of flaxseed consumption pointed out that the whole or ground flaxseed had more prominent hypotensive effects than oil or lignan (Khalesi et al., 2015). There is a limited number of studies focused on the effect of lignan on blood pressure, and review and meta-analysis articles often included all types of flaxseed lignan source.

Although ingestion of flaxseed provides beneficial effects on health, the exact mechanism

how SDG can work in the body is not fully understood. Animal studies suggest that the lignan reduces blood pressure by acting as an ACE inhibitor and/or guanylyl cyclase activator (Prasad, 2004; Prasad, 2013), but there are many factors to consider for how lignan works in the human body. Plant lignan faces barriers before being activated in the body. Plant lignan needs to be converted into animal lignan by gut microflora. At the human large intestine, secoisolarisinal diglucoside is converted to SECO and/or ED, EL. Therefore, an individual's gut health is a crucial step for lignan absorption in the body. Ingested SDG is absorbed into the digestive system and excreted as enterolactone and enterodiols, which reach steady state after consuming SDG 2-3 times a day for about 3 days (Kuijsten et al., 2005). Females tend to have faster absorption of enterolactone and faster excretion than men due to smaller blood volume (Kuijsten et al., 2005). In this study, participants consumed 0.9 mg SDG/kg body weight. It is notable that there was one participant who could not convert enterolactone to enterodiols, causing low bioavailability in the circulatory system (Kuijsten et al., 2005). This result suggests that the type of bacteria in the microflora population, which could be different between individuals, is crucial for SDG absorption. The study by Nessbit et al. (1999) also observed 2 out of 11 participants had little or no enterolactone after flaxseed supplement. Kuijsten et al.'s study (2005) concluded that about 40% of the ingested SDG was available in the body. Microflora health could be affected by background diet and age. Absorption of plant lignan has a large variation between individuals (Biagi et al., 2013). One of the factors that could affect gut bacteria health is a non-digestible carbohydrate, which selectively promotes the growth of certain bacteria (Puupponen-Pimia et al., 2004). Even though background diet pattern was investigated by retrospective food frequency questionnaires in the current study, whether the questionnaire was able to accurately capture each participant's diet pattern and gut microflora health, which could have affected lignan absorption and/or interaction with other food sources, is questionable. It is essential to monitor the serum

enterolignan level in intervention studies with flaxseed or lignan supplementation.

FLC supplementation for 8 weeks did not result in any significant reduction in LDL, TAG, total cholesterol, or increase in HDL. In the current study, change in lipid profile after the intervention was analyzed because lipid profile, such as level of the total cholesterol, HDL, LDL and TAG level are good indicators of cardiovascular disease risks, and is highly associated with atherosclerosis, which influences blood pressure. Thanks to SDG's structural similarity to oestrogen, an oestrogenic or anti-oestrogenic effect was thought to be one of the mechanisms of the health beneficial effect (Adolphe et al., 2010). Oestrogen has been known to increase HDL level and decrease LDL level, thus providing cardiovascular protection, particularly observed in pre-menopausal females (Barton, 2013). Improvement of lipid profile, such as lowering LDL or/and elevating HDL, can slow down the atherosclerosis process. Lignan supplementation in animal models persistently showed positive results for reducing blood lipid level, cholesterol level, and inflammatory markers during high-fat diets (Liu et al., 2015; Sun et al., 2016; Cho et al., 2004). Rabbits on a high cholesterol diet followed by SDG treatment could prevent progression of atherosclerosis lesions, while rabbits without SDG treatment showed worsening of atherosclerosis (Prasad, 2008). When rats were fed a high-fat diet, addition of lignan enriched flaxseed powder reduced total triglyceride and LDL cholesterol, while there was no difference between regular meal and regular meal + ground flaxseed, indicating that the improvement of lipid profile was from lignan (Park and Velasquez, 2012). Whether flaxseed or plant lignan supplementation is effective for lipid profile improvement in healthy individuals is still questionable. When 10 - 40 g of milled flaxseed was ingested daily by younger adults under 50 years old it did not alter the blood lipid profile (Edel et al., 2016). The milled flaxseed was included in a prepared muffin, and this dose was equivalent to 38 – 150 mg of SDG daily dose (Edel et al., 2016).

In a study targeting type-2 diabetic patients, 600 mg of SDG did not change any lipid profile after 3-month FLC ingestion (Barre et al., 2012). Considering the study from Edel (2016) included participants already using cholesterol-lowering agents such as statins and/or ezetimibe, fibrates, the SDG may have lowered the cholesterol level through an interaction with the drugs, possibly augmenting the lowering effect of the drugs. Even though some studies reported that SDG could improve lipid profiles, animal models showed an increase in serum cholesterol level after ingesting SDG (Prasad 1997). Even ingestion of SDG after a high cholesterol meal reduced the reduction of atherosclerosis risk without changing blood cholesterol level (Prasad, 1997).

While there was no significant positive change in lipid profiles from FLC supplementation, there was an unexpected result found from the placebo supplement group, which used whey protein. After 8 weeks intervention, there was a significant time x supplement interaction for HDL level, cholesterol:HDL ratio and total triglyceride favouring to the placebo group. Whey protein was used as a placebo supplement simply due to its powder form and convenience for preparation. Also, the participants had to take only 600 mg per day, and we expected they would not be affected because of the small serving size. There are several studies investigating the effect of whey protein on blood lipid profile. Whey protein was effective for reducing the total TAG level, and LDL level when it was combined with lycopene (Petyaev et al., 2012). In a study by Pal et al. (2010), sedentary obese, overweight individuals were given whey protein supplementation for 12 weeks. After this intervention, there was a significant reduction in total TAG level and LDL level in the whey protein group. LDL level was reduced from 3.31 mmol/L to 3.08 mmol/L, and total cholesterol was also reduced from 5.36 mmol/L to 4.97 mmol/L. TAG level was reduced from 1.07 mmol/L to 0.93 mmol/L as well (Pal et al., 2010). However, the present study only used a small amount of whey protein,

while this study asked the participants to ingest 60 g whey protein mix (27 g protein) daily for a longer period (12 months). Meta-analysis of whey supplementation showed that whey protein reduced total triacylglyceride, while there was no effect on total cholesterol, LDL, and HDL (Zhang et al., 2016). This study also indicated that the triacylglyceride reduction effect disappeared when the dose of whey protein was reduced below 30 g/day (Zhang et al., 2016). When prehypertensive and hypertensive participants were supplemented with lactotripeptide, which is rich in whey protein, systolic and diastolic blood pressure were reduced by 1.66 mmHg and 0.76 mmHg. 24-hour ambulatory systolic and diastolic blood pressures were reduced by 1.30 mmHg and 0.57 mmHg, respectively (Qin et al., 2013). In a study from Petyaev et al. (2012), 70 mg of whey protein complex with a combination of lycopene, which is derived from tomatoes, was administered to participants with prehypertension, and this intervention exerted systemic blood pressure reduction, improvement in lipid profiles, oxidative stress and inflammation parameters. This is noteworthy because this study used only 70 mg of whey protein per day during only a 1-month intervention. This is a smaller dose and shorter duration than the current study. Therefore, choosing whey protein as a placebo supplement was not an optimal choice due to its possible overlapping beneficial effect to the human body. Although the supplement x time interaction favoured the whey protein group, the change in HDL and TAG level favouring the whey protein group was still within the reference range (0.60-2.30 mmol/L for TAG; 0.90-2.40 mmol/L for HDL), and these values could vary day by day, depending on activity level and dietary habits. Therefore, to determine the effect of this small dose of whey protein on lipid profile, clinical studies with a longer period of intervention time may be needed.

After 8 weeks of walking intervention, there was no significant change in blood pressure or blood lipid profile. Undoubtedly, regular exercise provides numerous health benefits to a

wide range of the population. Especially in mid-age adults, regular exercise slows down the aging process by increasing vessel compliance, preventing thickening of the left ventricular wall and preventing muscle mass loss (Bamman et al., 2003; Tanaka et al., 2000; Arbab-Zadeh et al., 2004). Exercise is also a great method to manage a variety of risk factors, such as blood glucose, and lipid profile. However, the type or intensity of exercises to have beneficial effects has been argued in many studies. Among many types of exercises, walking exercise has been widely used as a regular exercise intervention because of its low cost, relatively easiness, and accessibility.. Aerobic exercises including walking have been shown to have blood pressure lowering effects (Whelton et al., 2002). One study suggested that walking intensity or duration itself may not be important for hypertensive individuals. Among 32 hypertensive subjects who walked more than 10,000 steps for 12 weeks irrespective of duration and intensity, 30 hypertensive subjects showed a reduction in SBP and DBP from 149.3/98.5 to 139.1/90.1 mmHg (Masakata et al., 2000). Walking > 9,500 steps per day for 32 weeks improved overweight subjects' weight (-2.4 kg), BMI (-0.8 kg/m²), body fat (-1.9%), waist circumference (-1.8 cm), and HDL (+3mg/dl) (Schneider et al., 2006). For older adults specifically, 7100~8000 steps/day translates into 30 minutes of moderate-to-vigorous physical activity (Tudor-Locke et al., 2011). An endurance exercise training intervention can significantly improve the blood lipid profile (i.e. increase HDL, reducing LDL) especially if dietary adjustment is performed concurrently (Leon and Sanchez, 2001). HDL has a cardiovascular protective effect, and when dietary fat ingestion is increased, HDL level also increase (Mensink and Katan, 1992). Increase in HDL was more pronounced when ingested fatty acids and carbohydrates were replaced with saturated fatty acids or polyunsaturated fatty acids, such as fish oil (Yanai et al. 2015). When endurance exercise training was introduced at the same time, reduction in HDL was attenuated (Leon and Sanchez, 2001).

The current study does not agree with the expected results for changes in lipid profiles with exercise training. After 8 weeks of walking intervention, waist circumferences of participants were significantly reduced, but the lipid profile did not show any improvement. Moreover, some of the lipid profiles moved to a different direction from what we expected. After 8 weeks of walking or stretching exercise intervention, the change in total cholesterol level favoured the stretching group. It was an unexpected result, considering the conventional belief that walking exercise is one of the first exercises suggested for those who seek a health benefit.

After 8 weeks of exercise intervention, there were benefits evident from stretching exercise compared to the walking exercise. When hourly data of ambulatory blood pressure was analyzed, there was a group x time interaction where the stretching group reduced nighttime diastolic blood pressure, from 67.6 ± 8.6 mmHg to 65.7 ± 11.2 , while walking group increased from 66.5 ± 7.4 mmHg to 70.5 ± 10.9 mmHg. Although it didn't reach statistical significance, group x time interaction for nighttime systolic blood pressure was close to $p = 0.05$ ($p = 0.052$), changing from 122.5 ± 12.3 mmHg to 119.1 ± 3.0 mmHg, while the nighttime systolic blood pressure of walking group changed from 124.3 ± 11.6 mmHg to 127.9 ± 9.4 mmHg. Stretching exercises provides beneficial effects on the cardiovascular system, especially directly on blood vessels. Nishiwaki et al. (2015), showed that 4 weeks of stretching exercise improved arterial stiffness in middle-aged males. Stretching exercise produces shear stress and mechanical stress on blood vessels, and this enhances the endothelial function of the vessel (e.g. NO production), causing vasodilation and further blood pressure reduction (Shinno et al., 2017). Tanaka et al. (2000) indicated that during short-term training, increased pulse pressure and mechanical stretching during exercise could modify the cross-link between collagen fibers, leading to improved compliance. Stretching exercise also has a

positive effect on heart rate variability, which is a potential predictor for cardiovascular disease (Farinatti et al., 2011). This is achieved through alteration of heart autonomic control, regulating sympathetic and parasympathetic discharges (Farinatti et al., 2011). In this study, stretching exercises of trunk and hamstrings were able to increase post-exercise vagal activity, leading to faster heart rate recovery (Farinatti et al., 2011).

Studies of yoga, which are composed of many static stretching actions, support not only an improvement in blood pressure, but also lipid profile (TC and VLDL) and lipid oxidation, as well as oxidative stress level in individuals with type II diabetes (Gordon et al., 2008). Although the mechanism of cardiovascular protection effect remains unclear, there is a hypothesis that the effect of yoga is similar to that of statin, which is associated with endothelial NO production, anti-atherogenic, and leukocyte adhesion inhibition effect (Sengupta, 2012). Eight weeks of stretching exercise targeted to postmenopausal females improved brachial blood pressure and hemodynamic parameters, as well as sympathetic vasomotor activity (Wong and Figueroa, 2014). Improvement in arterial stiffness can lead to improved general cardiovascular health since arterial stiffness increases as individual gets aged. Despite some of beneficial results from yoga studies, when a yoga program was given to healthy individuals, there was no improvement in endothelial function (Hunter et al., 2012).

There has been no in-vivo mechanism determining how stretching or static stretching could alter the condition of blood vessels, and the study results are mixed. The relationship between trunk flexibility and arterial stiffness is affected by sex, age, medical history, exercise mode, intensity, and volume (Nishiwaki et al., 2015). It is notable that the stretching exercise in the present study was more structured than walking exercise. Participants in the stretching exercise group were given a package of stretching exercises containing detailed instruction

with pictures, while participants in the walking group were given simpler instruction including target heart rate. Since self-measurement of heart rate could create large variation between individuals, despite the high compliance rate of walking exercise, participants might not have met the target intensity which can diminish the effect from the walking exercise.

The physical activity level of each participant was assessed using the PASE questionnaire. PASE score is valid and reliable since it is significantly correlated to self-assessed health status. The PASE score can assess the physical activity of older adults, especially those older than 65 years old. PASE score of each group in the current study was higher than previously reported scores of sedentary older adults without cardiovascular diseases (138.9 ± 70.4) or hypertension (139.2 ± 69.2) (Washburn et al. 1999). When comparing to the score of sedentary older adults between 55 and 64 years old (144.9) from Washburn et al.'s study (1999), participants in the current study had higher baseline PASE scores (Table 12), indicating that the participants in this study were not necessarily sedentary.

Although walking exercise is often suggested as a first-line exercise intervention due to its convenience and accessibility, the best recommendation may be regular exercise regardless of type. Regular physical exercise training has been commonly used as one of the simple lifestyle changes to manage hypertension, diabetes, obesity, and metabolic syndrome. For healthy individuals who have high blood pressure, 150 – 240 minutes per week of moderate intensity exercise such as walking is highly recommended, and this is superior to vigorous activity, such as running, swimming, and biking, for cost and safety (Cleroux et al., 1999). A study showed that regular exercise of the lower limbs could decrease systolic and diastolic blood pressure by 5-7 mmHg, and these changes were independent of weight loss, alcohol or salt intakes (Cleroux et al., 1999). Considering 12 mmHg of systolic blood pressure over 10 years could lower the risk of 1 death by cardiovascular disease in every 11 patients, reduction

in blood pressure caused by exercise could be clinically significant (Chobanian et al., 2003). It is important to maintain a regular exercise habit because the antihypertensive effect persists only if individuals keep doing exercise regularly. When they stopped for about 10 weeks, the antihypertensive effect disappeared (Cleroux et al., 1999). Therefore, maintaining regular exercise is crucial to protect against cardiovascular risks. Cardiovascular improvement by exercise also can entail a reduction in medication use, as shown in a study by Kokkinos et al. (2001). Physical activity can be beneficial to the vascular system by altering elastin, collagen, and smooth muscle tone. Structural changes in these elements are believed to occur over years.

It is noteworthy that the baseline lipid levels affect the lipid profiles change response to the exercise (Leon and Sanchez, 2001). According to this review, low baseline HDL was a predictor for positive HDL response to an exercise intervention, while the baseline LDL level was inversely associated with the response to an exercise intervention. Most of the participants from the current study had lipid profiles within the reference value, except for a few people. Therefore, walking exercise in the current study might not be sufficient to promote the lipid profile changes in the relatively “healthy” individuals.

Since walking exercise could be interpreted as a simple lifestyle modification activity which could be performed very easily, participants may have substituted the walking exercise intervention for their regular walking activity, possibly with lesser intensity required from the study. This behavior change might not be sufficient enough to enhance positive change in the cardiovascular parameters measured in this study. Also, participants were asked to maintain their regular lifestyle. Therefore, it is possible that many of the participants already had an active lifestyle, rather than being sedentary. Although many health-related organizations such as CSEP suggest 3 - 4 times a week for 30 minutes, there are opinions that this may not be

enough (CSEP, 2011). One study reported that, to have clinically significant improvement, the exercise should be prescribed at the frequency of 5 or more times per week (Perri et al., 2002). This study pointed that out that the monitoring of adherence to exercise intervention is crucial. We used self-report exercise log books to track the compliance of participants to exercise interventions, and there was a high compliance rate of over 89%. For walking exercise, using additional monitoring systems such as a mobile heart rate monitor could minimize the variation from self-measurement of heart rate. Although PASE has a certain degree of validity and reliability, the limitation of the recall-based questionnaires, such as individual differences in recall biases, has to be considered (Sallis & Saelens, 2000). Also, according to the meta-analysis by Lee et al. (2010), the mean length of the walking exercise necessary for reduction in blood pressure was 19 weeks. Many of the studies used moderate to high intensity for walking exercise and considering that the current study targeted otherwise healthy people, we might need a higher intensity and longer intervention period to promote positive changes.

Despite many studies promoting regular physical activity for improving the pre-mentioned conditions caused by sedentary lifestyle, according to a meta-analysis, non-structured or lifestyle activities have almost no effect on blood lipid profile (Leon and Sanchez, 2001). This could partially explain the problem from the current study, since we employed a non-supervised, free walking exercise program, which may not have sufficient exercise volume to cause positive changes in blood lipid profile, especially to those who were not necessarily sedentary in the past. Studies often focused on sedentary individuals, especially those with obesity, to assess the effect of exercises. The intensity in this study was moderate (50-65 % of estimated maximum heart rate), and other studies suggest that moderate to high intensity (65-85 % of maximum heart rate) are necessary to see significant blood pressure reduction (Lee

et al., 2010). When moderate (50-74 % of maximum heart rate) or high (75-84 % of maximum heart rate) intensity biking exercise was prescribed to a group of obese people, diastolic blood pressure in the high intensity exercise group was reduced significantly more than the diastolic blood pressure in the moderate intensity group (Kannan et al., 2014). A walking intervention with behavioral or cognitive-behavioral strategies was more successful for lowering blood pressure than one with simple instructions or prescription only (Lee et al., 2010). Therefore, additional strategies, such as using pedometers could help participants to achieve a targeted goal by enhancing interests and motivation, especially when the intervention is given unsupervised.

For this study, instead of a single time blood pressure measurement using the conventional sphygmomanometer, an automated ambulatory blood pressure was used. This device repeatedly measures an individual's blood pressure for a given period (e.g. 24 hours) with a specific frequency (e.g. every 20 minutes). Ambulatory blood pressure data could be superior to traditional single time blood pressure measurement for predicting the mortality caused by cardiovascular diseases (Dolan et al., 2005). Conventional single time blood pressure measurement has limitations for assessing patients' "true" blood pressure because of the phenomena of white coat hypertension or masked hypertension. Because this study targeted older adults over 50 years old, and white coat hypertension is common in this age group, using the ambulatory blood pressure monitor helped to eliminate possible errors during the intervention. Even though there was no significant difference between on-site measured blood pressure and 24h ambulatory blood pressure (data not shown) in this study, there were 3 cases of exclusion from the study at the screening process, due to normotensive readings from the ambulatory BP monitor despite readings of high blood pressure at the office measurement. Ambulatory blood pressure data could reflect true real-life blood pressure,

which includes resting, active, and sleeping blood pressure data. Ambulatory blood pressure monitoring can provide multiple data points during the investigation period. These multiple data points can provide additional information that could not be obtained from a conventional sphygmomanometer, such as dipper and non-dipper classification, nocturnal blood pressure, and blood pressure variability. It can monitor change in blood pressure over time after administering certain agents or after acute exercise sessions, and this could be valuable in clinical trials involving pharmaceutical or supplementary agents, or exercise interventions.

In the present study, FLC supplementation and walking exercise interventions for 8 weeks could not produce beneficial effects for either blood pressure or blood lipid/glucose parameters. There are several possible reasons why these results deviated from previous studies. Flaxseed and flax lignan are known not only to reduce blood pressure, but also to improve various cardiovascular parameters such as blood lipid and glucose profile, and oxidative stress indicators. Milled flax supplementation to PAD patients for 12 months reduced total blood cholesterol and LDL cholesterol level by 15 % when it was administered with cholesterol-lowering medication such as statin, and the subgroup who did not take cholesterol-lowering medication, but flaxseed only, also showed reduced circulating cholesterol level (Edel et al., 2015). This 1-year study reported that the cholesterol-lowering effect from flaxseed was the largest in the first 6 months, and the effect was attenuated thereafter (Edel et al., 2015). Even though baseline cholesterol level in the current study was somewhat higher than the pre-mentioned study (5.6 – 5.9 mmol/L vs 4.4 - 4.6 mmol/L), our FLC intervention did not show any positive change. Considering the component difference between FLC in the current study and milled flaxseed in the Edel et al. study, the fat component of the flaxseed might have helped to lower the cholesterol. However, in the other study (Fukumitsu et al. 2010), 100 mg of SDG lowered LDL/HDL ratio level relative to the

placebo group after 12 weeks. Although it didn't reach the statistical significance, the SDG group showed a reduction of total and LDL cholesterol by 6.2% and 8.4% respectively (Fukumitsu et al. 2010). Despite its lower SDG dose than the current study (100 mg vs 600 mg), subjects had a similar level of baseline serum cholesterol without taking any cholesterol-lowering agent. Fukumitsu et al. (2008) suggested that SDG could decrease mRNA level of sterol regulatory element binding protein-1 (SREBP-1), which regulates the activity of cholesterol and fatty acid synthesis.

Many studies administrated FLC or flaxseed supplements for more than 6 months, compared to 8 weeks (~2 months) in the current study. According to a meta-analysis, the effect of blood pressure reduction was greater when the intervention was longer than 12 months (Ursoniu et al., 2015). The study from Cornish et al. (2009) had a very similar study protocol compared to the present study. This study showed a significant reduction in diastolic pressure of male participants and metabolic syndrome score after 6 months of exercise and FLC supplementation intervention (Cornish et al., 2009). Cornish et al. (2009) also had a larger sample size (100 vs 23) and used different placebo (maltodextrin as a capsule vs whey protein as a powder). In the study by Stuglin and Prasad (2005), fifteen healthy male participants were given flaxseed supplementation for 4 weeks. The supplement intervention did not show any significant change in systolic and diastolic blood pressure, as well as blood cholesterol level. However, this study showed a significant increase in total TG level with flaxseed supplements. The effect of flaxseed supplementation on serum lipid in human is very variable and, generally, normocholesterolemic human adults do not show a change in serum lipid (Stuglin and Prasad, 2005). Mean blood pressure of participants in this study was 124.0/78.3, which is a normal range of blood pressure, while the present study only used the subjects with blood pressure over 130/85 mmHg. In the FLAX-PAD study (Caligiuri et al.

2016), 110 patients with peripheral arterial disease were given flax supplement for over a year. The flaxseed supplementation reduced central systolic blood pressure by 3.4 mmHg and 4.9 mmHg, central diastolic blood pressure by 1.3 mmHg and 2.6 mmHg after 6 and 12 months (Caligiuri et al. 2016). In contrast, central DBP of the placebo group increased 1.1 mmHg and 1.5 mmHg at 6 and 12 months (Caligiuri et al. 2016). Considering the relatively short period of time (8 weeks) for the supplementation intervention in the present study, the effect of flaxseed supplementation could be time-sensitive, or need a certain length of intervention period, e.g. > 6 months. The meta-analysis also stated that the benefit from blood pressure reduction was greater when the flaxseed supplement was consumed longer than 12 months (Khalesi et al., 2015). It should be noted that, in this analysis, the ground flaxseed, flax oil, defatted flax, and flax lignan were not differentiated. Fourteen studies were analyzed in this article, and 4 studies used specifically flax lignan (Khalesi et al., 2015). The FLAX-PAD study, which is the largest clinical trial with flaxseed supplementation, used food containing the flaxseed equivalent to the dose of 30 g/day (Leyva et al., 2011).

The present study only included participants who were not taking any anti-hypertensive agents or supplements, so that we could solely investigate the effect of flax lignan, SDG. In the FLAX-PAD study, 110 of participants were subjected to SDG supplementation, regardless of taking anti-hypertensive agents, and showed 1.3 mmHg reduction in systolic and 1.1 mmHg in diastolic blood pressure after 6 months (Caligiuri et al. 2016). What is noted at the end of this study was that there could have been an interaction between the antihypertensive agent and supplement. While the biochemical mechanisms of SDG are not fully understood, the effective dose and duration of flaxseed or SDG supplementation need to be studied more closely.

The current study has several limitations. The number of participants who completed the

interventions is very low ($n = 25$). To fulfill the desired power at 80%, one hundred participants were required as mentioned in the methods section. Lipid profile and blood pressure have very high variability between individuals, therefore, a larger number of participants is needed because of this inter-individual variation. According to Statistics Canada, only 4% of hypertensive people aged between 20 and 79 years old are untreated and 14% of same age group are unaware of their condition (Statistics Canada, 2016, <https://www150.statcan.gc.ca/n1/pub/82-625-x/2016001/article/14657-eng.htm>). This reflects a very narrow window for recruitment in the current study, possibly leading to low recruitment rate.

Although both whey protein and FLC did not change blood pressure, choosing the placebo agent needs to be done with more caution. As shown in the results, whey protein used as placebo produced some positive effect on lipids. Also, using human participants has many confounding factors, such as regular physical activity patterns and dietary patterns, which are difficult to control. Due to the low number of participants, the distribution of the participants to each experimental arm was not even; for example, there were only 2 people in the exercise-supplement group. The blocks were created initially for a total 60 participants, but the recruitment was lower than what we expected. Therefore, we had to analyze the data from each intervention separately, not considering the interaction between two distinct interventions. A more structured exercise program may need to be employed, with further individualized protocols. Participants' daily physical activity patterns were not modified. They were asked to maintain their physical activity levels, and simply add walking exercise on top of that. If participants had additional exercise activities other than walking exercise (e.g. cycling, swimming, etc.), the effect of walking exercise could be masked. Due to the lack of monitoring and supervision for exercise intervention other than a logbook, it is

questionable if the exercise was executed correctly at the target intensity. Many of the participants already had awareness and interest in reducing blood pressure without medication. Their dietary habits were not controlled during the interventions, but according to the food frequency questionnaires, there were no significant nutritional differences between the groups. The macronutrient distribution of CHO, fat, and protein after the intervention were about 42%, 41%, and 17%, respectively. CHO and protein did not differ much from the recommendation from Health Canada, but fat consumption was above the acceptable distribution range: 45-65%, 10-35%, and 20-35% for CHO, fat, and protein respectively (Health Canada, 2011). These limitations are more pronounced due to the low number of participants. This study recruited participants during different seasons for 2 years. This could have a substantial effect on the results in both dietary and exercise interventions. For example, participants who started interventions during winter and those who started during the summer season may have different exercise conditions and different response to exercise. Dietary habit might be altered depending on the season, especially when the intervention period is during the holiday season such as Christmas or Thanksgiving. Blood lipid level becomes higher in colder seasons than in warmer seasons (Gordon et al. 1987). Since we did not control participants' regular eating and physical activity habits, this could lead to extra variation in measurements.

Despite a possible antihypertensive effect (nighttime DBP) in the stretching exercise group, the change was minimal, and it is questionable if the change was clinically significant. For example, a 30 mmHg of reduction in clinic SBP (equivalent to 10 mmHg of reduction in 24h ambulatory BP) reduced the risk of CV death by 0.2% in 5-year-follow-up in the Dublin Outcome study (Dolan et al., 2005). In the PAMELA study, a 30 mmHg of reduction in clinic SBP reduced 11-year-risk of CV death by 5% (Sega et al., 2005). In addition, to reduce the

risk of 11-year-risk CV death by ~4%, a 10 mmHg of reduction in nighttime DBP was needed (Sega et al., 2005). The change in nighttime DBP in the current study was only ~ 2 mmHg.

Even though supplement and exercise interventions were administrated concurrently, the current study analyzed two interventions separately due to the low number of participants. However, it is still possible that participants in each intervention group had confounding effect from another intervention. Therefore, the change observed in supplement groups could be partially affected by exercise intervention, and vice versa. The results should be interpreted with caution.

Chapter 4: Conclusion

In summary, this double-blinded randomized clinical study attempted to target otherwise healthy older adults with high normal blood pressure or stage I hypertension. After 8 weeks, FLC supplementation and walking exercise did not result in any improvement in systolic and diastolic blood pressure, blood glucose, TG, HDL, LDL, and CRP level. However, using a small amount of whey protein as placebo generated unexpected time x supplement interactions for TG and HDL level, as well as cholesterol:HDL ratio in favour of the placebo group. Moreover, there was time x exercise interactions for nighttime diastolic blood pressure and nighttime mean arterial pressure in favour of the stretching group. However, the change observed in this study is minimal and is not satisfying minimal clinically significant differences. Therefore, the results in the current study should be interpreted with caution. Studying participants with FLC or flaxseed supplementation, while maintaining regular life style, for a longer period (> 6 months) may be needed, and this could provide valuable and clinically significant information regarding managing blood pressure in an older age group. Simple supplementation and adding physical exercise such as walking may not be sufficient to promote improvement in cardiovascular parameters, especially for a short period such as 2 months, and further food adjustment and individualized and structured exercise program may be needed to see clinically significant improvements.

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Appendix A Consent Form Flaxseed Lignan Study

Participant Information and Consent Form

TITLE: Flaxseed lignan enriched complex for blood pressure reduction in elderly participants with high normal blood pressure or stage I hypertension

PRINCIPAL STUDY INVESTIGATORS:

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Terra Arnason, MD, PhD, FRCPC Adult Endocrinology, Department of Medicine, College of Medicine, University of Saskatchewan, (306) 844-1119.

Student Researchers:

Jong Ko, B.Sc., and Vania Araujo Guirado, undergraduate student (College of Kinesiology, supervised by Philip Chilibeck), Shanal de Silva, M.Sc. (College of Pharmacy and Nutrition, supervised by Jane Alcorn).

SPONSOR: Saskatchewan Health Research Foundation

EMERGENCY TELEPHONE NUMBER: (306) 242-0868

INTRODUCTION

You are invited to take part in this research study that involves combining daily BeneFlax® (flaxseed lignan enriched complex) supplementation with exercise (walking or stretching) in men and women who are 50 years of age or older. This study is being conducted by researchers at the University of Saskatchewan to evaluate whether the flaxseed lignan enriched complex can reduce slightly elevated blood pressure to normal values. Your participation is voluntary. It is up to you to decide whether or not you wish to take part. If you decide to participate, you are still free to withdraw at any time and without giving any reasons for your decision. If you do not wish to participate, you will not affect your relationship with the researchers or the university.

Please take time to read the following information carefully. You can ask the study staff to explain any information that you do not clearly understand. You may ask as many questions as you need. Please feel free to discuss this with your family, friends or family physician before you decide.

WHO IS CONDUCTING THE STUDY?

The study is sponsored by the University of Saskatchewan with a grant funded by the Saskatchewan Health Research Foundation. The Saskatchewan Health Research Foundation will reimburse the investigators and university for the cost of undertaking this study. However, neither the institution nor any of the investigators or staff will receive any direct financial benefit from conducting this study.

WHY IS THIS STUDY BEING DONE?

Current recommendations today suggest that use of therapeutic lifestyle changes, such as supplementation with specific dietary components and moderate exercise, are appropriate interventions for individuals with borderline high blood pressure. Dietary intervention with natural products, such as flaxseed, is associated with decreased prevalence of cardiovascular disease. Lignans are the active compounds in flaxseed believed to mediate the cardiovascular health benefits following flaxseed consumption. Lignans are chemical compounds found in many plants but flaxseed, in particular, contains very high levels of these chemicals. Clinical trials indicate benefit of Flax Lignan Enriched Complex (FLC) (BeneFlax® from Archer Daniels Midland, a natural product derived from flaxseed composed of 34-38% lignan) in reductions in blood cholesterol and glucose. Furthermore, a recent study in atherosclerosis patients showed significant clinical benefit of flaxseed meal in reductions of blood pressure. As a therapeutic lifestyle change that could prevent a need to introduce drug therapy in elderly patients, the effectiveness of FLC supplementation alone or in combination with moderate exercise merits investigation.

The primary objective is to examine a dietary intervention with 600 mg/day of the flaxseed lignan, secoisolariciresinol diglucoside, in the form of BeneFlax® with either a walking exercise program or a stretching exercise program for their effects on blood pressure (and other cardiovascular risk factors) in participants of age 50 years or greater with borderline high blood pressure.

WHO CAN PARTICIPATE IN THIS STUDY?

You are eligible to participate in this study if you are a male or female aged 50 years or older, can walk unaided for 30 minutes, and have borderline high blood pressure (high normal blood pressure (130/85 – 139/89) or stage I hypertension (140/90 – 159/99)). We require that your physician be contacted and made aware of your involvement in the study.

You cannot take part in the study if you have taken medications for blood pressure control in the past 12 months. Further, you are not eligible to participate if you are at risk of low blood pressure; have diabetes; have blood pressure values >140/90 mmHg with macrovascular target organ damage as defined by the Canadian Hypertension Education Program 2014; have history of stroke, cerebrovascular disease or coronary artery disease and do not wish to refrain from taking any other natural health products (except vitamin supplements) one week prior to and during

the duration of the intervention; have cancer or have been diagnosed with cancer within the last two years; have heart, liver, kidney, stomach or intestine disorders; have intolerances to flax seed; are currently taking a flax seed supplement; or have participated in any other clinical trial with an investigational agent within one month of starting this trial. You will also be excluded if you have taken oral antibiotics in the past three months. We will determine whether exercise training is safe for you by having you fill out a brief questionnaire (the Physical Activity Readiness Questionnaire and Physical Activity Scale for the Elderly). If there is doubt about the safety of exercise participation for you based on these questionnaires, we will need to get permission from your family physician before you can participate in this study. You may not participate in the study if you do not consent to having your physician be contacted and made aware of your involvement in the study or you do not have a regular family physician.

WHAT DOES THE STUDY INVOLVE?

Dosing - Participants will receive either: 1) SDG-enhanced (38%) food grade flax lignan marketed in Canada as BeneFlax® (Archer Daniel Midlands, Natural Health Products File # OF2-31-3-13412-2-4.) at a dose of 300 milligrams [438 µmoles] SDG twice per day, which is contained in 0.8 grams of BeneFlax®, or 2) Whey protein (Natural Factors, Whey Factors unflavoured) 0.3 grams twice daily. The whey protein is being used as a “placebo” comparator and we do not expect it to have any effects on blood pressure. Participants will also be randomized to do walking exercise or flexibility exercise training 5 times per week for 30-60 minutes per session.

1. Screening visit

Before you can be enrolled into the study several screening procedures are necessary to ensure that you are eligible to participate. We will enquire about your medical history, medication use, and age and you will undergo an abbreviated physical to ensure that you are healthy to participate. As well, we will measure your lying and standing blood pressure and ask you to complete a physical activity assessment questionnaire.

2. Assignment to a treatment group

If you agree to participate in the study you will be randomized (i.e. assigned by chance, by a computer) into one of 4 groups: Group 1 will do a flexibility program (stretching exercises) five times per week and consume 300 mg of placebo (whey protein) twice a day with a meal; Group 2 will do an exercise program (walking for 30-60 minutes) five times per week and consume 300 mg of placebo twice a day with a meal; Group 3 will do a flexibility program (stretching exercises) five times per week and consume 800 mg of flaxseed lignan enriched complex (FLC) product (BeneFlax®) twice a day with a meal; Group 4 will do an exercise program (walking for 30-60 minutes) five times per week and consume 800 mg of FLC twice a day with a meal. A “placebo” is something that looks like the FLC but has no active

ingredients. It is necessary to use a placebo in this type of experiment to determine the effectiveness of the FLC. You will not know whether you receive the FLC or placebo and neither will the investigators (i.e. the study will be double blind). This can be determined, however, in case of emergency. You will have an equal chance of being assigned to one of the four groups. The chance of being assigned to the placebo treatment, placebo exercise group is 25%.

3. Administration of treatments

You will take the FLC at a dose of 300 milligrams lignan (SDG) (which is contained in 0.8 grams of BeneFlax®) or placebo (0.3 grams of whey protein) twice a day with a meal for 8 weeks. You should take the treatment at the same time of the day (e.g. always with breakfast or always with supper).

The exercise training will be done five times per week for 8 weeks. If you are in the exercise program you will walk for 30-60 minutes (you can start with 30 minutes and increase this to 60 minutes as you proceed through the trial) that will be supervised by study research personnel. The walking program will be conducted at the Williams Building (University of Saskatchewan, 221 Cumberland Ave North, Room 108) using treadmills or outside along the Meewasin Trail (meet at the Physical Activity Complex, University of Saskatchewan) depending upon weather conditions. If you are in the flexibility program you will be given a flexibility program that will involve stretching all the major muscle groups. We will give you an orientation session for the flexibility program (Williams Building gym location (RJD Williams Building at the University of Saskatchewan, 221 Cumberland Ave North, Room 108) and then this program will be continued at home unsupervised. It is expected that training sessions will take about an hour each. All participants will be asked to follow the 'Dash Eating Plan' (a diet that limits salt, sweets, sugary beverages, and red meat intake, and emphasizes vegetables, fruits, low fat dairy, whole grains, fish, poultry, beans and nuts). Information about this eating plan will be provided to all participants before the start of the study.

4. Study measurements

Measurements of blood pressure will be done at baseline, the midpoint of the study (week 4) and end of the intervention (week 8). Measurements of vital signs, body weight and height, physical activity levels and diet, a whole-body scan with dual energy X-ray absorptiometry (DXA), a 6-minute walking test, as well as blood samples will be taken at baseline, and at the end of the intervention (week 8). Overnight fasting is required for the blood measurements. You will be able to eat immediately following the blood sampling. You will have to stop eating at 10:00 pm the night before, although you may consume water. The following tests or measurements will be done:

- 1) Blood pressure – we will measure your resting blood pressure using a blood pressure cuff as well as provide you with an ambulatory blood pressure monitor that monitors your blood pressure over a 24 h period. This will be done three times (baseline, week 4, week 8) during the study.

- 2) Vital signs – heart rate and respiratory rate will be measured. Your height and body weight and waist circumference will also be measured.
- 3) Body composition – we will determine your total body composition (e.g. fat content) by use of dual energy x-ray absorptiometry (DXA). This involves assessment of lean tissue and fat mass with dual energy X-ray absorptiometry by a nuclear medicine technologist in the RJD Williams Building at the University of Saskatchewan (221 Cumberland Ave North, Room 108). You will need to lie on a table while you are scanned with an X-ray. This test takes about 10 minutes.
- 4) A six-minute walk test. This test requires that you walk at a brisk pace for six minutes. You are scored for performance on this test by how far you can walk in six minutes.
- 5) Blood collection – You will be asked to provide blood on three occasions (at the start, half-way and at the end). The amount of blood will be 35 mL, which is about 2 tablespoons. A certified Laboratory Technologist will collect the blood samples and this will occur at the University of Saskatchewan. Your blood will be tested for indicators of oxidative stress and inflammation, blood lipids (e.g. cholesterol) and glucose, and for a screen of cells and molecules that indicate the health of your organs. These molecules let the researchers know how the body is affected by aging and then how the body is affected after a person takes the flaxseed lignan. Note that your blood will not be kept after the study for future uses unrelated to this study.
- 6) We will give you questionnaires about your diet and physical activity levels. These questionnaires take about 45 minutes to complete.
- 7) We will ask you to maintain a daily log to track whether you took the study product and performed your exercise training each day and to indicate a potential adverse event like a change in bowel movement. We will also ask you to keep all empty and full study product packets and return these at the end of the study.

We will do all measurements mentioned in one session and the total time for these measurements will be about 1 hour.

A total of 100 participants will be enrolled in this study at the University of Saskatchewan.

WHAT ARE MY RESPONSIBILITIES?

As a study participant you will be expected to do the following:

1. Over the 8 weeks of the study we ask that you consume the study product provided to you by the study research personnel and adhere to the exercise training program.
2. We ask that you allow us to monitor your blood pressure, heart rate, respiratory rate, body weight, total body composition, and waist circumference three times over the 8 week study.
3. We ask that you keep a daily log of your study product use and exercise training, as well as document any relevant side effects.
4. Allow us to contact your family physician.

WHAT ARE THE BENEFITS OF PARTICIPATING IN THIS STUDY?

You may decrease your blood pressure and you may increase your flexibility and cardiovascular health by participating in this study. These benefits are not guaranteed. You will also help to establish potentially useful information about the benefit of flaxseed lignan enriched complex in blood pressure control in individuals over the age of 50 years.

WHAT ARE THE POSSIBLE RISKS AND DISCOMFORTS?

From previous clinical studies we have conducted with the flaxseed lignan enriched complex, no side effects have been reported. However, it is possible to experience a change in bowel movement (e.g. loose stool) due to the slight increase in fibre content.

The exercise may result in muscle strains or soreness.

There is a small amount of radiation exposure from the dual energy X-ray scans for total body composition measurement. This is equal to one tenth of the amount of radiation you would receive from taking a trans-Atlantic flight from North American to Europe, or less than 0.5% from what you would receive from a routine full-mouth dental X-ray.

The risks of drawing blood include temporary discomfort from needle stick, bruising, and, in rare cases, infection.

Your blood will be screened for metabolites. Any abnormalities known to the researchers which might reasonably have impact on your health and well-being will be discussed with your physician, and information will be provided to you if deemed appropriate by the physician.

The study is “double-blind” meaning that neither the participant nor the researchers know the treatment group assignments. However, that information will be made available in the event of a medical emergency.

WHAT ARE ALTERNATIVES TO THE STUDY?

You do not have to participate in this study to receive a supervised exercise training program or BeneFlax®. Supervised training programs are available at different gyms in the city and Flaxseed products (which are not lignan enriched) are widely available.

WHAT HAPPENS IF I DECIDE TO WITHDRAW?

Your participation in this research is voluntary. You may withdraw from this study at any time. You do not have to provide a reason. Your relationships with the researchers or the university will not be affected. However, you will be asked to return to study personnel all of the supplements you were given.

If you choose to enter the study and then decide to withdraw at a later time, all data collected about you during your enrolment and up to the time of your withdrawal will

be retained for analysis. At the end of the study we can tell you which study treatment group you were in.

WHAT HAPPENS IF SOMETHING GOES WRONG?

In the case of a medical emergency related to the study, you should seek immediate care and, as soon as possible, notify the principal investigator. Inform the medical staff you are participating in a clinical study. Necessary medical treatment will be made available at no cost to you. By signing this document, you do not waive any of your legal rights against the sponsor, investigators or anyone else.

WHAT HAPPENS AFTER COMPLETION OF THE STUDY?

We will inform you of the overall study results and your individual results (if desired) after we have analyzed all data.

WHAT WILL THE STUDY COST ME?

You will not be charged for the study product or any research-related procedures. You will not be paid for participating in this study. Reimbursement for study-related expenses (e.g. travel, parking, meals) is not available.

WILL MY PARTICIPATION BE KEPT CONFIDENTIAL?

In Saskatchewan, the Health Information Protection Act (HIPA) defines how the privacy of your personal health information must be maintained so that your privacy will be respected. Your name will not be attached to any information, nor mentioned in any study report, nor be made available to anyone except the research team. It is the intention of the research team to publish results of this research in scientific journals and to present the findings at related conferences and workshops, but your identity will not be revealed.

Information derived from the study will be kept for 25 years and Health Canada may have access to the information (in accordance with Part 4 of the Natural Health Products Regulations).

WHO DO I CONTACT IF I HAVE QUESTIONS ABOUT THE STUDY?

If you have any questions or desire further information about this study before or during participation, you can contact Jane Alcorn at (306) 966-6365 (or jane.alcorn@usask.ca) or Philip Chilibeck at (306) 966-1072 (or phil.chilibeck@usask.ca).

If you have any concerns about your rights as a research participant and/or your experiences while participating in this study, contact the Chair of the University of Saskatchewan Research Ethics Board, at 306-966-4053 (out of town calls 1-888-966-4053). The Research Ethics Board is a group of individuals (scientists, physicians, ethicists, lawyers and members of the community) that provide an independent review of human research studies. This study has been reviewed and approved on ethical grounds by the University of Saskatchewan Research Ethics Board.

CONSENT TO PARTICIPATE

- I have read (or someone has read to me) the information in this consent form.
- I understand the purpose and procedures and the possible risks and benefits of the study.
- I have been informed of the alternatives to the study.
- I was given sufficient time to think about it.
- I had the opportunity to ask questions and have received satisfactory answers.
- I am free to withdraw from this study at any time for any reason and the decision to stop taking part will not affect my future relationships at the university.
- I agree to follow the principal investigator's instructions and will tell the principal investigator at once if I feel I have had any unexpected or unusual symptoms.
- I have been informed there is no guarantee that this study will provide any benefits to me.
- I give permission for the use and disclosure of my de-identified personal health information collected for the research purposes described in this form.
- I understand that by signing this document I do not waive any of my legal rights.
- I will be given a signed and dated copy of this consent form.
- My family physician can be informed about my participation in this study, and, if required, consulted regarding my health and treatment.
 - ☐ Yes, you may contact my primary care physician

- I agree to participate in this study:

Printed name of participant: _____

Signature _____ Date_____

Printed name of person obtaining consent: _____

Signature _____ Date_____

Appendix B Walking Exercise Prescription for Flaxseed Lignan Study

Instruction

- **Supplementation : daily ingestion, 2 times per day**
- **Exercise : 5 days per week**
- **Walking between 30 and 60 minutes per day**
- **How hard should I walk?**
 - **50% - 65% of your predicted maximal heart rate**
 - ◆ **Predicted maximal heart rate**

$$= 220 - \text{age} = \underline{\hspace{2cm}} \text{ bpm}$$

- ◆ $\underline{\hspace{2cm}} \text{ bpm} \times 0.5 = \underline{\hspace{2cm}} \text{ bpm}$
- ◆ $\underline{\hspace{2cm}} \text{ bpm} \times 0.65 = \underline{\hspace{2cm}} \text{ bpm}$

- **Use your radial or carotid pulse to count the number of beats in a 15 second time span**
- $\underline{\hspace{2cm}} \text{ beats in 15 seconds} \times 4 = \underline{\hspace{2cm}} \text{ bpm}$
- **Take your pulse count approximately 10 min into your exercise session and toward the end of your exercise session**
- **If intensity was found to be too low or too high, feel free to modify your walking speed**
- **Please record the amount of time you spend for walking exercise in minutes**

Appendix C Stretching Exercise Prescription for Flaxseed lignan study

Flexibility Training Program

By Denise Cornish BSPE, PFLC

Flexibility- defined as the range of motion (ROM) available in a joint or a group of joints. ROM is specific to each joint. It is determined by factors such as joint structure, connective tissue (i.e. skin, tendons, ligaments, etc), muscle imbalances, muscle bulk, activity level, age and gender. It is recommended that one should participate regularly in a flexibility training program in order to improve joint range of motion.

The term: “use it or lose it” is very accurate in describing your body’s range of motion. If a joint is immobilized or not used throughout its full ROM the connective tissue will loose its extensibility and become stiff. However, participating in a regular flexibility program will assist you in maintaining the best possible ROM.

The purpose of flexibility training is to lengthen your muscles and loosen the joint and its connective tissue so that you will develop an optimal level of flexibility allowing for efficient movement. In other words, you will develop the ability to move with freedom in order to do the everyday movements that we often take for granted. Other benefits to flexibility training may include: muscular relaxation, stress relief, improvement in your bodily appearance through improved posture, relief of low back pain and possible injury prevention.

Do’s and Don’ts

Do warm-up before stretching with an activity that will get your heart pumping for a few minutes (i.e. walking, marching on the spot, stair-climbing, etc.)

Do breath comfortably during the stretch. Preferably take natural deep breaths that allow your abdomen (not your chest) to rise up and down during each breath.

Do move slowly and smoothly to find the point of *mild* tension. Then, hold the stretch until the tension releases (approx. 10-60 seconds).

Don’t bounce during the stretch. Avoid sudden or jerky movements that may elicit the stretch reflex causing the muscle to contract rather than relax and lengthen.

Do allow your body to relax – use deep breathing, soft music, a mat or folded towel, etc.

Do maintain a positive attitude throughout your session.

Do wear comfortable loose fitting clothing that will allow for movement

Do schedule your exercise into your daily plan in order to maintain your motivation and commitment. Perhaps try exercising at the same time every day.

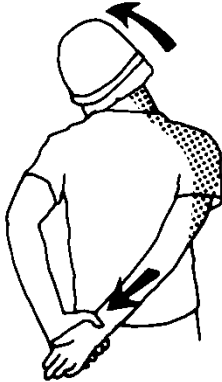
Do keep a record of your exercise. Place a check mark on a calendar for each day that you have stretched. Look at the calendar frequently to encourage you that you have been keeping on track or for a visual reminder to stretch more frequently.

Do remember: Improvements in your ROM are gradual and do not occur over night.

Version Date: October 26, 2004

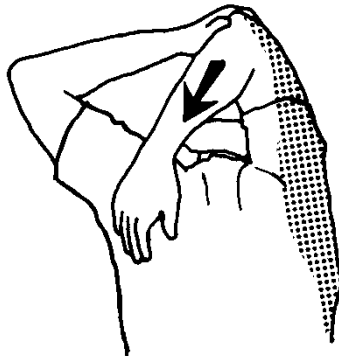
Flexibility Exercises

Remember: Warm-up before stretching. Breathe comfortably during the stretch. Move slowly and smoothly to find the point of *mild* tension. Then, hold the stretch until the tension releases (approx. 10-60 seconds). Don't bounce during the stretch.



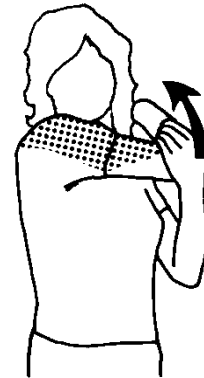
Lateral Neck

Hold arm behind your back. Lean head away toward the opposite shoulder. Hold 30 seconds. Repeat with other side.



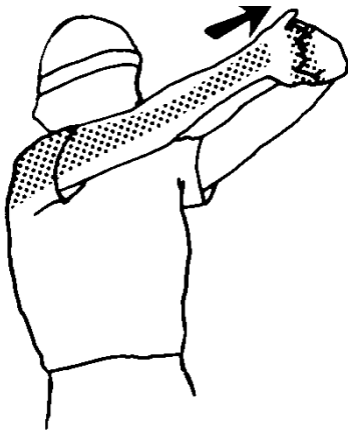
Inferior Shoulder

Place arm overhead as shown. Use opposite arm to pull elbow in toward the mid-line of your body. Hold 30 seconds. Repeat with other arm.



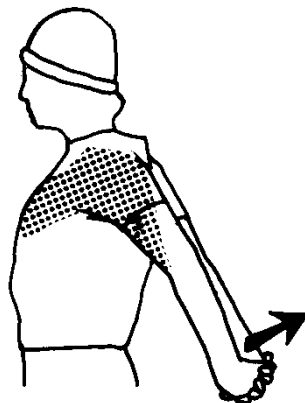
Posterior Shoulder

Place arm across body as shown. Use other hand to pull elbow in to opposite shoulder. Hold 30 seconds. Repeat with other arm.



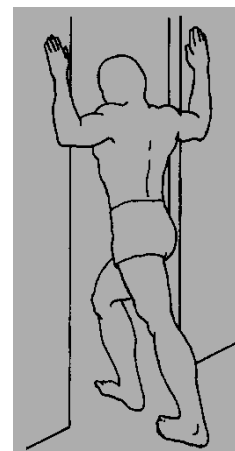
Shoulder Protraction

Interlace fingers and press arms forward in order to round out your upper back. Hold 30 seconds.



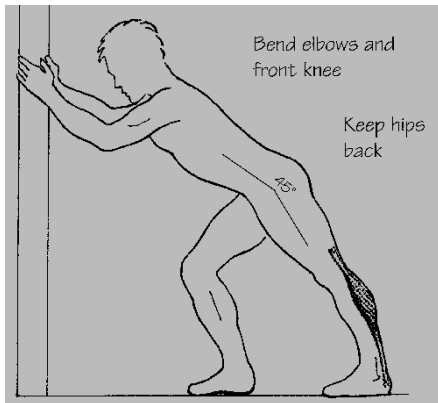
Shoulder Extension

Lift arms up behind your back as far as comfort allows. Do not lean forward – Keep tall. Hold 30 seconds.



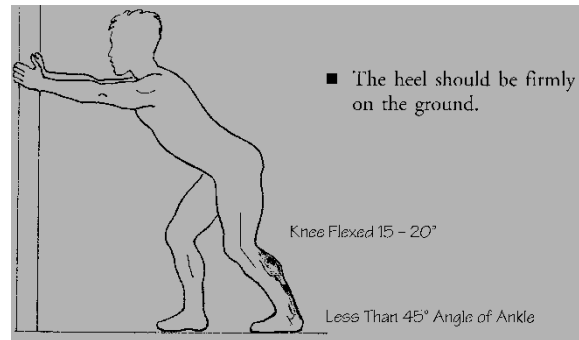
Chest (Pectoralis)

Stand in front of a doorway with hands placed above head. Lean into the frame until you feel a stretch. Hold 30 seconds.



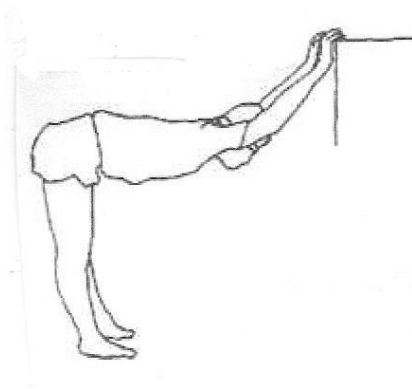
Calf (Gastronemius)

Stand with hands on wall for support. Step back with one leg and press the heel firmly into the ground. Bend front leg and arms to lean the body forward until you feel tension in the calf. Hold 30 seconds. Repeat with other leg.



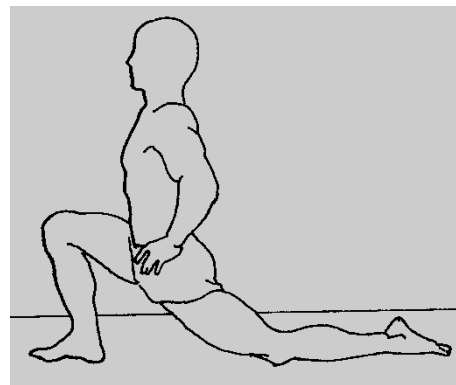
Calf (Soleus)

The positioning for the soleus stretch is the same as the gastrocnemius stretch, except the knee must be bent. Heel should remain firmly against the ground. Hold 30 seconds. Repeat with other leg.



Shoulders and Back

Stand with hands on ledge in front of you. Slowly bend over at the hips until you feel a stretch in shoulders and back. Hold for 30 seconds.



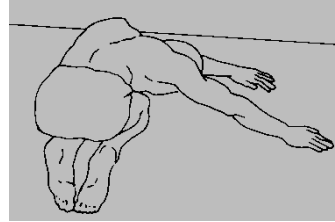
Hip Flexor – kneeling

Kneel on one leg with the other leg bent in front for support. Keep body tall. Slightly shift body weight forward until you feel a stretch down the front of the hip and thigh. Hold 30 seconds. Repeat with other leg.



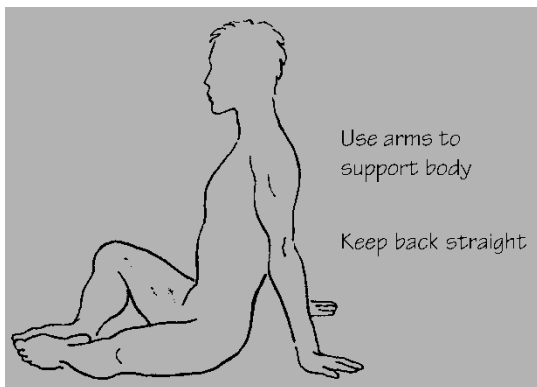
Back (Lat Dorsi)

Begin kneeling on all fours. Sit back on to your heels. Slowly walk your hands forward while allowing your head and chest to relax. Hold 30 seconds.



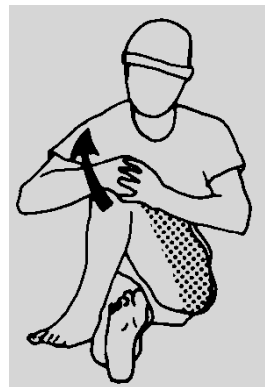
Back and Side (Quadratus Lumborum)

The positioning for the back and side stretch is like the previous back stretch, except reach hands over to one side before walking them forward. Hold 30 seconds.



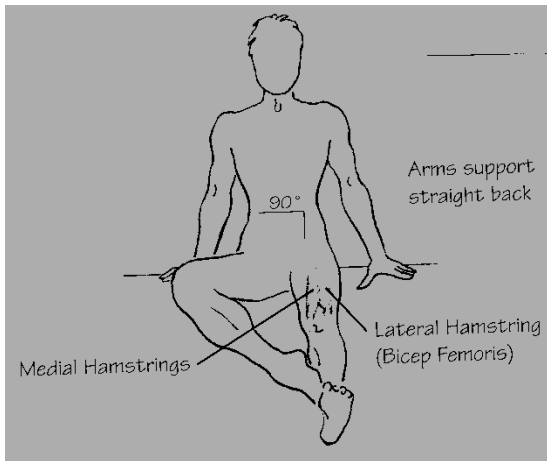
Groin (Adductor) – seated

Sit tall with back against a wall or use hands for support as shown. Place soles of feet together. Slowly slide heels toward body until you feel a stretch in your groin. Hold 30 seconds.



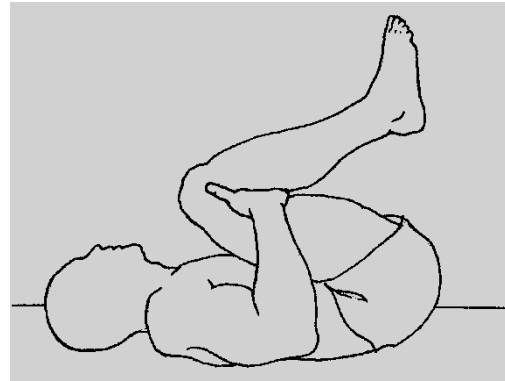
Hip (Piriformis) - seated

Sit tall and cross one leg over the other. Use hands to pull the knee across body toward opposite shoulder. Hold 30 seconds. Repeat with other leg.



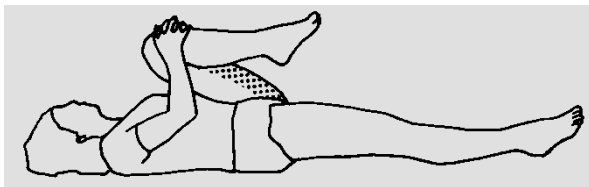
Hamstrings – seated

Sit tall with back against a wall or use hands for support as shown. Bend one knee and allow it to fall to the side. Keep the other leg straight at a 90° angle to the pelvis with toes pointing up. Lean forward from the hips slightly for more stretch (if needed).



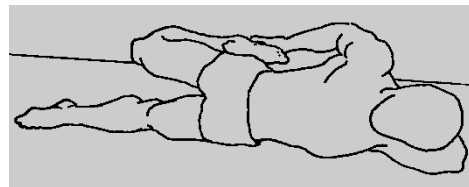
Back - Double knee tuck

Lie on back and tuck both knees into chest. Hold 30 seconds.



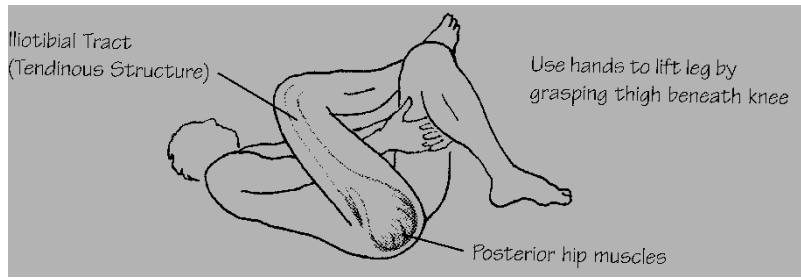
Back - Single knee tuck

Lie on back and tuck one leg into chest. Allow other leg to lie straight and relax. Hold 30 seconds. Repeat with other leg.



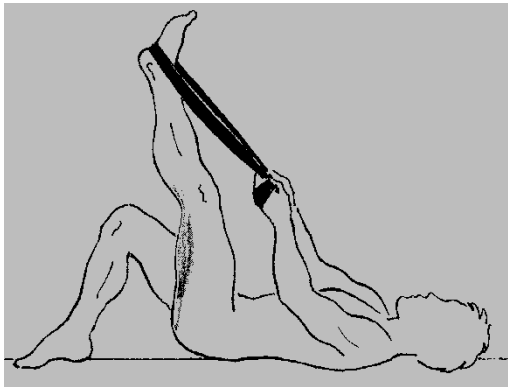
Quadriceps – side lying

Lie on side. Head can rest on bottom arm. Bend knee of the top leg and hold above the ankle. Keep thigh in line with body. Squeeze buttocks and pelvic tilt. Pull back on leg for more stretch (if needed). Hold 30 seconds. Do other leg.



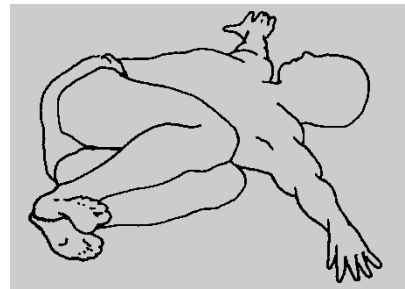
Posterior Hip and Thigh (Gluteus)

Lie on back and cross leg. If more stretch is needed, use hands to gently pull leg towards body (use a towel behind the thigh if you must lift upper back off the floor in order to reach the thigh). Hold 30 seconds. Repeat with other leg.



Hamstrings – Lying

Lie on back with one leg straight and the other leg bent with foot flat on floor. Grasp the ends of a towel placed in the arch of your foot (like a stirrup) OR use hands to grasp your leg for support. Gently pull the leg towards your head until you feel a stretch behind the thigh. Hold 30 seconds. Repeat with other leg.



Spinal Rotators

Lie on back with knees bent and arms out to the side for support. Slowly lower legs to the floor on one side allowing your back to rotate. Keep elbows, head and shoulders on the floor. Hold 30 seconds. Repeat on other side.

Appendix D Supplementation and exercise tracking log

The Effect of Flaxseed and Exercise to blood pressure



Funded By:



Saskatchewan
Health Research
FOUNDATION

**University of
Saskatchewan**

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Phone: 966-1072
E-mail: phil.chilibeck@usask.ca

Supplement and Exercise Tracking Log Book

Place a (✓) in the appropriate box to verify that you have taken your Supplement. Place a (✓) on the day that you completed your exercise. Write Date as shown below. Please contact 966-1072 or 717-0398 if you have any questions or concerns.

Week 1		Date: April 15—21, 2013
Monday April 15	Supplement	✓
	Exercise	✓
		Comment

Name: _____

Week ____	Date:	
Monday	Supplement	Comment
	Exercise	
Tuesday	Supplement	Comment
	Exercise	
Wednesday	Supplement	Comment
	Exercise	
Thursday	Supplement	Comment
	Exercise	
Friday	Supplement	Comment
	Exercise	
Saturday	Supplement	Comment
	Exercise	
Sunday	Supplement	Comment
	Exercise	

Week ____	Date:	
Monday	Supplement	Comment
	Exercise	
Tuesday	Supplement	Comment
	Exercise	
Wednesday	Supplement	Comment
	Exercise	
Thursday	Supplement	Comment
	Exercise	
Friday	Supplement	Comment
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Saturday	Supplement	Comment
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Sunday	Supplement	Comment
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Week ____	Date:	
Monday	Supplement	Comment
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Tuesday	Supplement	Comment
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Saturday	Supplement	Comment
	Exercise	
Sunday	Supplement	Comment
	Exercise	

Week ____	Date:	
Monday	Supplement	Comment
	Exercise	
Tuesday	Supplement	Comment
	Exercise	
Wednesday	Supplement	Comment
	Exercise	
Thursday	Supplement	Comment
	Exercise	
Friday	Supplement	Comment
	Exercise	
Saturday	Supplement	Comment
	Exercise	
Sunday	Supplement	Comment
	Exercise	